518 Rec'd PCT/PTO 0 1 AUG 2001

FORM PTO-1390
(REV. 11-2000)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER
205502-9004

U S. APPLICATION NO. (If known, see 37 CFR 1 5
09/890604

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/00074	January 31, 2000	February 1, 1999

TITLE OF INVENTION
Modification of Lignin Composition of Gymnosperms

APPLICANT(S) FOR DO/EO/US
ELLIS, David Dunham; CHAPPLE, Clinton Charles Spencer; GILBERT, Margarita

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [XX] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [XX] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [XX] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [XX] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [XX] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [XX] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [XX] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [XX] Other items or information: **See attached sheet.**

JC17 Rec'd PCT/PTO 01 AUG 2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/890604	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER

	CALCULATIONS	PTO USE ONLY
21. [X] The following fees are submitted: **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... **$1000.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... **$860.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... **$710.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... **$690.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	54 - 20 =	34	x **$18.00**	$ 612.00	
Independent claims	9 - 3 =	6	x **$80.00**	$ 480.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$ 270.00	

	CALCULATIONS	PTO USE ONLY
TOTAL OF ABOVE CALCULATIONS =	$ 2,222.00	
[X] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +	$ 1,111.00	
SUBTOTAL =	$ 1,111.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$ 1,111.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$	
TOTAL FEES ENCLOSED =	$ 1,111.00	
	Amount to be refunded:	$
	charged:	$

a. [X] A check in the amount of $ 1,111.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. ____________ in the amount of $ __________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 12-0064 . A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

MICHAEL BEST & FRIEDRICH LLC
401 N. Michigan Ave., Suite 1700
Chicago, IL 60611

Lisa C. Childs
SIGNATURE

Lisa C. Childs
NAME

39937
REGISTRATION NUMBER

JC17 Rec'd PCT/PTO 01 AUG 2001

IN THE
UNITED STATES PATENT & TRADEMARK OFFICE 09/890604

IN RE APPLICATION OF: ELLIS, et al.	Art Group Unit:
DOCKET NO.: 205502-9004	Examiner:
Based on International Publication No. WO 00/46382 Published August 10, 2000 International Application No. PCT/CA00/00074 Filed January 31, 2000 Priority Date Claimed: February 1, 1999	FIRST PRELIMINARY AMENDMENT
FILED ON: HEREWITH	
FOR: Modification of Lignin Composition of Gymnosperms	

TO: ASSISTANT COMMISSIONER OF PATENTS
WASHINGTON, D.C. 20231

Dear Sirs:

[X] AUTHORIZATION TO PAY AND PETITION FOR THE ACCEPTANCE OF ANY NECESSARY FEES: If any charges or fees must be paid in connection with the following Communication (including but not limited to the payment of issue fees), they may be paid out of our deposit account No. 12-0064. If this payment also requires a Petition, please construe this authorization to pay as the necessary Petition which is required to accompany the payment.

[ ] Applicant herewith petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office Action dated ____________ for ____________month(s) from ____________ to ____________. Submitted herewith is check No. ________ for $____________ to cover the cost of the extension. If a check is lost, or otherwise does not accompany this Petition, please charge my deposit account number 12-0064 in the appropriate amount to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to the above numbered deposit account.

FIRST PRELIMINARY AMENDMENT

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on:
Date: ____________

Signature:____________
Print:

MICHAEL, BEST & FRIEDRICH L.L.C.
401 N. Michigan Avenue, Suite 1700
Chicago, IL 60611-2412
Telephone: (312)661-2100 Facsimile: (312) 661-0029

Attorney Docket No. 205502-9004
Serial No. NOT YET ASSIGNED

FIRST PRELIMINARY AMENDMENT

Please cancel claims 10 and 12.

Respectfully submitted,

Date: 8/1/01

Lisa C. Childs

Lisa C. Childs
Atty. Registration No. 39937
MICHAEL BEST & FRIEDRICH LLC
401 N. Michigan Avenue, Suite 1700
Chicago, IL 60611-2412
Phone: (312)661-2100
Fax: (312)661-0029

S:\CLIENT\205502\9004\C0025887

JC05 Rec'd PCT/PTO 01 MAR 2002

#3

IN THE
UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF:	ELLIS, et al.	)	
CASE:	205502-9004	)	**RESPONSE TO:**
INT'L APPL'N NO.	PCT/CA00/00074	)	**NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DO/EO**
INT'L FILING DATE	31 JAN 2000	)	
U.S. SERIAL NO.:	09/890,604	)	
FILED ON:	01 AUG 2001	)	
FOR:	Modification of Lignin Composition of Gymnosperms	)	

BOX: PCT
ASSISTANT COMMISSIONER OF PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, DC 20231

Dear Sirs:

[X] AUTHORIZATION TO PAY AND PETITION FOR THE ACCEPTANCE OF ANY NECESSARY FEES: If any charges or fees must be paid in connection with the following Communication (including but not limited to the payment of issue fees), they may be paid out of our deposit account No. 50-1965. If this payment also requires a Petition, please construe this authorization to pay as the necessary Petition which is required to accompany the payment.

[x] Applicant herewith petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office Action dated October 2, 2001 for three (3) month(s) from December 2, 2001 to March 2, 2002. Submitted herewith is check No. 12612 which includes the $ 460.00 extension fee. If a check is lost, or otherwise does not accompany this Petition, please charge my deposit account number 50-1965 in the appropriate amount to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to the above numbered deposit account.

EXPRESS MAIL LABEL NO. EL 874 050 187 US

Date of Deposit March 1, 2002

I hereby certify that the above listed papers or fees are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above, addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231. The person mailing these papers/fees is:

SIGNATURE ______________
Michael A. Finnegan

MICHAEL BEST & FRIEDRICH LLC
401 North Michigan Avenue, Suite 1900
Chicago, Illinois 60611-4212
(312) 661-2100 Fax: (312) 661-0029

RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DO/EO

This is in response to the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States DO/EO dated October 2, 2001.

Applicant hereby submits the Oath and Declaration, signed by each of the inventors, along with check number 12612 in the amount of $ 525.00 which includes the required surcharge of $ 65.00.

Accompanying this submission is Applicants' Sequence Listing in computer readable form as required underr 37 C.F.R. 1.821(e).

STATEMENT UNDER 37 C.F.R. 1.821(e-g), 1.825(b), and 1.825 (d)

Applicant hereby states that the Sequence Listing submitted hereto as Exhibit A is recorded in computer readable form, that it is identical to the Sequence Listing submitted herewith on 3.5" floppy diskette, and that it does not include new matter which goes beyond the disclosure in the International Application.

Respectfully submitted,

Lisa C Childs 3/1/2002

Charles A. ~~Laff~~
Reg. No. 19787
Lisa C. Childs
Reg. No. 39937
LAFF, WHITESEL & SARET, LTD.
401 North Michigan Avenue, Suite 1700
Chicago, IL 60611
(312) 661-2100
(312) 222-0818 (fax)

Agents for Applicant

03/07/2002 MNGUYEN 00000073 09890604

01 FC:254 65.00 OP
02 FC:217 460.00 OP

S:\CLIENT\205502\9004\C0082794

ENTERED

PCT09

RAW SEQUENCE LISTING DATE: 03/22/2002
PATENT APPLICATION: **US/09/890,604** TIME: 11:40:36

Input Set : **A:\41193--1.txt**
Output Set: **N:\CRF3\03222002\I890604.raw**

```
     3 <110> APPLICANT: Ellis, David D
     4       Chapple, Clinton C S
     5       Gilbert, Margarita
     7 <120> TITLE OF INVENTION: Modification of Lignin Composition of Gymnosperms
     9 <130> FILE REFERENCE: 41193-B
    11 <140> CURRENT APPLICATION NUMBER: 09/890,604
C--> 12 <141> CURRENT FILING DATE: 2002-03-01
    14 <150> PRIOR APPLICATION NUMBER: US 60/118,124
    15 <151> PRIOR FILING DATE: 1999-02-01
    17 <150> PRIOR APPLICATION NUMBER: PCT/CA00/00074
    18 <151> PRIOR FILING DATE: 2000-01-31
    20 <160> NUMBER OF SEQ ID NOS: 2
    22 <170> SOFTWARE: PatentIn version 3.1
    24 <210> SEQ ID NO: 1
    25 <211> LENGTH: 5156
    26 <212> TYPE: DNA
    27 <213> ORGANISM: Arabidopsis thaliana
    29 <220> FEATURE:
    30 <221> NAME/KEY: CDS
    31 <222> LOCATION: (2487)..(3002)
    32 <223> OTHER INFORMATION:
    35 <220> FEATURE:
    36 <221> NAME/KEY: CDS
    37 <222> LOCATION: (3902)..(4519)
    38 <223> OTHER INFORMATION:
    41 <220> FEATURE:
    42 <221> NAME/KEY: CDS
    43 <222> LOCATION: (3131)..(3556)
    44 <223> OTHER INFORMATION:
    47 <400> SEQUENCE: 1
    48 aagcttatgt atttccttat aaccatttta ttctgtatat agggggacag aaacataata     60
    50 agtaacaaat agtggtttta tttttttaaa tatacaaaaa ctgtttaacc attttatttc    120
    52 ttggttagca aaattttgat atattcttaa gaaactaata ttttaggttg atatattgca    180
    54 gtcactaaat agttttaaaa gacacgaagt tggtaagaac aggcatatat tattcgattt    240
    56 aattaggaat gcttatgtta atctgattcg actaattaga aacgacgata ctatgagctc    300
    58 atagatggtc ccacgaccca ctctcccatt tgatcaatat tcaactgagc aatgaaacta    360
    60 attaaaaacg tggttagatt aaaaaaataa attgtgcagg tagcggatat ataatactag    420
    62 taggggttaa aaataaaata aaacaccaca gtattaaatt tttgtttcaa aagtattatc    480
    64 aatagttttt ttgcttcaaa aatatcacaa atttttgtat gaaatatttc tttaacgaaa    540
    66 ataaattaaa taaaatttaa aatttatatt tggagttcta tttttaattt agagttttta    600
    68 ttgttaccac attttttgaa ttattctaat attaatttgt gatattatta caaaaagtaa    660
    70 aaatatgata ttttagaata ctattatcga tatttgatat tattgacctt agctttgttt    720
    72 gggtggagac atgtgattat cttattacct ttttattcca tgaaactaca gagttcgcca    780
```

```
RAW SEQUENCE LISTING                          DATE: 03/22/2002
PATENT APPLICATION:  US/09/890,604            TIME: 11:40:36

Input Set : A:\41193--1.txt
Output Set: N:\CRF3\03222002\I890604.raw

 74 ggtaccatac atgcacacac cctcgtgaag ccgtgactta atatgatcta gaacttaaat    840
 76 agtactacta attgtgtcat ttgaactttc tcctatgtcg gtttcacttc atgtatcgca    900
 78 gaacaggtgg aatacagtgt ccttgagttt cacccaaatc ggtccaattt tgtgatatat    960
 80 attgcgatac agacatacag cctacagagt tttgtcttag cccactggtt ggcaaacgaa   1020
 82 attgtcttta tttttttatg ttttgttgtc aatgtgtctt tgtttttaac tagattgagg   1080
 84 tttaatttta atacatttgt tagtttacag attatgcagt gtaatctgat aatgtaagtt   1140
 86 gaactgcgtt ggtcaaagtc ttgtgtaacg cactgtatct aaattgtgag taacgacaaa   1200
 88 ataattaaaa ttaaaggacc ttcaagtatt attagtatct ctgtctaaga tgcacaggta   1260
 90 ttcagtaata gtaataaata attacttgta taattaatat ctaattagta aaccttgtgt   1320
 92 ctaaacctaa atgagcataa atccaaaagc aaaaatctaa acctaactga aaaagtcatt   1380
 94 acgaaaaaaa gaaaaaaaaa agagaaaaaa ctacctgaaa agtcatgcac aacgttcatc   1440
 96 ttggctaaat ttatttagtt tattaaatac aaaaatggcg agtttctgga gtttgttgaa   1500
 98 aatatatttg tttagccact ttagaatttc ttgttttaat ttgttattaa gatatatcga   1560
100 gataatgcgt ttatatcacc aatattttg ccaaactagt cctatacagt catttttcaa   1620
102 cagctatgtt cactaattta aaacccactg aaagtcaatc atgattcgtc atatttatat   1680
104 gctcgaattc agtaaaatcc gtttggtata ctatttattt cgtataagta tgtaattcca   1740
106 ctagatttcc ttaaactaaa ttatatattt acataattgt tttctttaaa agtctacaac   1800
108 agttattaag ttataggaaa ttatttcttt tatttttttt tttttttagg aaattatttc   1860
110 ttttgcaaca catttgtcgt ttgcaaactt ttaaaagaaa ataaatgatt gttataattg   1920
112 attacatttc agtttatgac agattttttt tatctaacct ttaatgtttg tttccctgtt   1980
114 tttaggaaaa tcataccaaa atatatttgt gatcacagta aatcacggaa tagttatgac   2040
116 caagattttc aaagtaatac ttagaatcct attaaataaa cgaaatttta ggaagaaata   2100
118 atcaagattt taggaaacga tttgagcaag gatttagaag atttgaatct ttaattaaat   2160
120 attttcattc ctaaataatt aatgctagtg gcataatatt gtaaataagt tcaagtacat   2220
122 gattaatttg ttaaaatggt tgaaaaatat atatatgtag attttttcaa aaggtatact   2280
124 aattattttc atattttcaa gaaaatataa gaaatggtgt gtacatatat ggatgaagaa   2340
126 atttaagtag ataatacaaa aatgtcaaaa aaagggacca cacaatttga ttataaaacc   2400
128 tacctctcta atcacatccc aaaatggaga actttgcctc ctgacaacat ttcagaaaat   2460
130 aatcgaatcc aaaaaaaaca ctcaat atg gag tct tct ata tca caa aca cta    2513
131                              Met Glu Ser Ser Ile Ser Gln Thr Leu
132                              1               5
134 agc aaa cta tca gat ccc acg acg tct ctt gtc atc gtt gtc tct ctt     2561
135 Ser Lys Leu Ser Asp Pro Thr Thr Ser Leu Val Ile Val Val Ser Leu
136 10                  15                  20                  25
138 ttc atc ttc atc agc ttc atc aca cgg cgg cga agg cct cca tat cct     2609
139 Phe Ile Phe Ile Ser Phe Ile Thr Arg Arg Arg Arg Pro Pro Tyr Pro
140                 30                  35                  40
142 ccc ggt cca cga ggt tgg ccc atc ata ggc aac atg tta atg atg gac     2657
143 Pro Gly Pro Arg Gly Trp Pro Ile Ile Gly Asn Met Leu Met Met Asp
144             45                  50                  55
146 caa ctc acc cac cgt ggt tta gcc aat tta gct aaa aag tat ggc gga     2705
147 Gln Leu Thr His Arg Gly Leu Ala Asn Leu Ala Lys Lys Tyr Gly Gly
148         60                  65                  70
150 ttg tgc cat ctc cgc atg gga ttc ctc cat atg tac gct gtc tca tca     2753
151 Leu Cys His Leu Arg Met Gly Phe Leu His Met Tyr Ala Val Ser Ser
152     75                  80                  85
154 ccc gag gtg gct cga caa gtc ctt caa gtc caa gac agc gtc ttc tcg     2801
155 Pro Glu Val Ala Arg Gln Val Leu Gln Val Gln Asp Ser Val Phe Ser
156 90                  95                  100                 105
```

RAW SEQUENCE LISTING DATE: 03/22/2002
PATENT APPLICATION: **US/09/890,604** TIME: 11:40:36

Input Set : **A:\41193--1.txt**
Output Set: **N:\CRF3\03222002\I890604.raw**

```
158 aac cgg cct gca act ata gct ata agc tat ctg act tac gac cga gcg      2849
159 Asn Arg Pro Ala Thr Ile Ala Ile Ser Tyr Leu Thr Tyr Asp Arg Ala
160                 110                 115                 120
162 gac atg gct ttc gct cac tac gga ccg ttt tgg aga cag atg aga aaa      2897
163 Asp Met Ala Phe Ala His Tyr Gly Pro Phe Trp Arg Gln Met Arg Lys
164             125                 130                 135
166 gtg tgt gtc atg aag gtg ttt agc cgt aaa aga gct gag tca tgg gct      2945
167 Val Cys Val Met Lys Val Phe Ser Arg Lys Arg Ala Glu Ser Trp Ala
168         140                 145                 150
170 tca gtt cgt gat gaa gtg gac aaa atg gtc cgg tcg gtc tct tgt aac      2993
171 Ser Val Arg Asp Glu Val Asp Lys Met Val Arg Ser Val Ser Cys Asn
172     155                 160                 165
174 gtt ggt aag ctacttcaca tattcaccac tcttgctata tatatgtgca              3042
175 Val Gly Lys
176 170
178 attaaacaaa tatgtaaaaa gtgaaagtac tcatttcttc tttctttagt atgtacttta    3102
180 acatttaacc aaaacaattg taggtaag cct ata aac gtc ggg gag caa att       3154
181                                Pro Ile Asn Val Gly Glu Gln Ile
182                                            175                 180
184 ttt gca ctg acc cgc aac ata act tac cgg gca gcg ttt ggg tca gcc      3202
185 Phe Ala Leu Thr Arg Asn Ile Thr Tyr Arg Ala Ala Phe Gly Ser Ala
186                 185                 190                 195
188 tgc gag aag gga caa gac gag ttc ata aga atc tta caa gag ttc tct      3250
189 Cys Glu Lys Gly Gln Asp Glu Phe Ile Arg Ile Leu Gln Glu Phe Ser
190             200                 205                 210
192 aag ctt ttt gga gcc ttc aac gta gcg gat ttc ata cca tat ttc ggg      3298
193 Lys Leu Phe Gly Ala Phe Asn Val Ala Asp Phe Ile Pro Tyr Phe Gly
194         215                 220                 225
196 tgg atc gat ccg caa ggg ata aac aag cgg ctc gtg aag gcc cgt aat      3346
197 Trp Ile Asp Pro Gln Gly Ile Asn Lys Arg Leu Val Lys Ala Arg Asn
198     230                 235                 240
200 gat cta gac gga ttt att gac gat att atc gat gaa cat atg aag aag      3394
201 Asp Leu Asp Gly Phe Ile Asp Asp Ile Ile Asp Glu His Met Lys Lys
202 245                 250                 255                 260
204 aag gag aat caa aac gct gtg gat gat ggg gat gtt gtc gat acc gat      3442
205 Lys Glu Asn Gln Asn Ala Val Asp Asp Gly Asp Val Val Asp Thr Asp
206                 265                 270                 275
208 atg gtt gat gat ctt ctt gct ttt tac agt gaa gag gcc aaa tta gtc      3490
209 Met Val Asp Asp Leu Leu Ala Phe Tyr Ser Glu Glu Ala Lys Leu Val
210             280                 285                 290
212 agt gag aca gcg gat ctt caa aat tcc atc aaa ctt acc cgt gac aat      3538
213 Ser Glu Thr Ala Asp Leu Gln Asn Ser Ile Lys Leu Thr Arg Asp Asn
214         295                 300                 305
216 atc aaa gca atc atc atg gtaattatat ttcaaaaagc actagtcata             3586
217 Ile Lys Ala Ile Ile Met
218     310
220 gtcatgtttc ttaatgcgtt acgtaataat acttatccat tgaccagtta ttttctccta    3646
222 agttttttg tttgaattag gaaggtaatt ttctatttta ctagagaaag caacagattt     3706
224 tagcatgatc tttttttaat atatatagaa gcattgaata ttcagatcta caataattat    3766
```

RAW SEQUENCE LISTING DATE: 03/22/2002
PATENT APPLICATION: **US/09/890,604** TIME: 11:40:36

Input Set : **A:\41193--~1.txt**
Output Set: **N:\CRF3\03222002\I890604.raw**

```
gaaactaatg aagagacaaa aaatggagag agaaaaaaga aagagtggac tagtgtggat   3826
atatttaatt ctaatttgat tttattagga cgttatattt aattctaatt tgattttttt   3886
atttgatttt attag gac gtt atg ttt gga gga acg gaa acg gta gcg tcg     3937
                 Asp Val Met Phe Gly Gly Thr Glu Thr Val Ala Ser
                 315                 320                 325
gcg ata gag tgg gcc tta acg gag tta tta cgg agc ccc gag gat cta      3985
Ala Ile Glu Trp Ala Leu Thr Glu Leu Leu Arg Ser Pro Glu Asp Leu
            330                 335                 340
aaa cgg gtc caa caa gaa ctc gcc gaa gtc gtt gga ctt gac aga cga      4033
Lys Arg Val Gln Gln Glu Leu Ala Glu Val Val Gly Leu Asp Arg Arg
        345                 350                 355
gtt gaa gaa tcc gac atc gag aag ttg act tat ctc aaa tgc aca ctc      4081
Val Glu Glu Ser Asp Ile Glu Lys Leu Thr Tyr Leu Lys Cys Thr Leu
    360                 365                 370
aaa gaa acc cta agg atg cac cca ccg atc cct ctc ctc ctc cac gaa      4129
Lys Glu Thr Leu Arg Met His Pro Pro Ile Pro Leu Leu Leu His Glu
375                 380                 385                 390
acc gcg gag gac act agt atc gac ggt ttc ttc att ccc aag aaa tct      4177
Thr Ala Glu Asp Thr Ser Ile Asp Gly Phe Phe Ile Pro Lys Lys Ser
                395                 400                 405
cgt gtg atg atc aac gcg ttt gcc ata gga cgc gac cca acc tct tgg      4225
Arg Val Met Ile Asn Ala Phe Ala Ile Gly Arg Asp Pro Thr Ser Trp
            410                 415                 420
act gac ccg gac acg ttt aga cca tcg agg ttt ttg gaa ccg ggc gta      4273
Thr Asp Pro Asp Thr Phe Arg Pro Ser Arg Phe Leu Glu Pro Gly Val
        425                 430                 435
ccg gat ttc aaa ggg agc aat ttc gag ttt ata ccg ttc ggg tcg ggt      4321
Pro Asp Phe Lys Gly Ser Asn Phe Glu Phe Ile Pro Phe Gly Ser Gly
    440                 445                 450
cgt aga tcg tgc ccg ggt atg caa cta ggg tta tac gcg ctt gac tta      4369
Arg Arg Ser Cys Pro Gly Met Gln Leu Gly Leu Tyr Ala Leu Asp Leu
455                 460                 465                 470
gcc gtg gct cat ata tta cat tgc ttc acg tgg aaa tta cct gat ggg      4417
Ala Val Ala His Ile Leu His Cys Phe Thr Trp Lys Leu Pro Asp Gly
                475                 480                 485
atg aaa cca agt gag ctc gac atg aat gat gtg ttt ggt ctc acg gct      4465
Met Lys Pro Ser Glu Leu Asp Met Asn Asp Val Phe Gly Leu Thr Ala
            490                 495                 500
cct aaa gcc acg cgg ctt ttc gcc gtg cca acc acg cgc ctc atc tgt      4513
Pro Lys Ala Thr Arg Leu Phe Ala Val Pro Thr Thr Arg Leu Ile Cys
        505                 510                 515
gct ctt taagtttatg gttcgagtca cgtggcaggg ggtttggtat ggtgaaaact       4569
Ala Leu
    520
gaaaagtttg aagttgccct catcgaggat ttgtggatgt catatgtatg tatgtgtata   4629
cacgtgtgtt ctgatgaaaa cagatttggc tctttgtttg cccttttttt tttttctttt   4689
aatggggatt ttccttgaat gaaatgtaac agtaaaaata agattttttt caataagtaa   4749
tttagcatgt tgcaaagatc gatcttggat gagaacttct acttaaaaaa aaaaaaaaaa   4809
ttttttttta gttatttcac ctttttcttt tgttctggtt gtatggttgc cattgtgtca   4869
```

RAW SEQUENCE LISTING DATE: 03/22/2002
PATENT APPLICATION: **US/09/890,604** TIME: 11:40:36

Input Set : **A:\41193--1.txt**
Output Set: **N:\CRF3\03222002\I890604.raw**

296 attaggggct ggaagttcgc tggttaaggc taaatcagag ttaaagttat aattttacaa 4929
298 gcccaacaaa aggtcgcaga ttaaaaccac atgatattta taaaaaaaat tctaaggttt 4989
300 ttattagttt tattttcagt ttactgagta ctatttactt ttttattttt tgcaaataaa 5049
302 tgtattttat catatttatg ttttttgtta taaactccaa acatacaggt ttcattacct 5109
304 aaaaaaagac agagtggttt cgttaatttt gtttcattaa tctcgag 5156
307 <210> SEQ ID NO: 2
308 <211> LENGTH: 520
309 <212> TYPE: PRT
310 <213> ORGANISM: Arabidopsis thaliana
312 <400> SEQUENCE: 2
314 Met Glu Ser Ser Ile Ser Gln Thr Leu Ser Lys Leu Ser Asp Pro Thr
315 1 5 10 15
318 Thr Ser Leu Val Ile Val Val Ser Leu Phe Ile Phe Ile Ser Phe Ile
319 20 25 30
322 Thr Arg Arg Arg Arg Pro Pro Tyr Pro Pro Gly Pro Arg Gly Trp Pro
323 35 40 45
326 Ile Ile Gly Asn Met Leu Met Met Asp Gln Leu Thr His Arg Gly Leu
327 50 55 60
330 Ala Asn Leu Ala Lys Lys Tyr Gly Gly Leu Cys His Leu Arg Met Gly
331 65 70 75 80
334 Phe Leu His Met Tyr Ala Val Ser Ser Pro Glu Val Ala Arg Gln Val
335 85 90 95
338 Leu Gln Val Gln Asp Ser Val Phe Ser Asn Arg Pro Ala Thr Ile Ala
339 100 105 110
342 Ile Ser Tyr Leu Thr Tyr Asp Arg Ala Asp Met Ala Phe Ala His Tyr
343 115 120 125
346 Gly Pro Phe Trp Arg Gln Met Arg Lys Val Cys Val Met Lys Val Phe
347 130 135 140
350 Ser Arg Lys Arg Ala Glu Ser Trp Ala Ser Val Arg Asp Glu Val Asp
351 145 150 155 160
354 Lys Met Val Arg Ser Val Ser Cys Asn Val Gly Lys Pro Ile Asn Val
355 165 170 175
358 Gly Glu Gln Ile Phe Ala Leu Thr Arg Asn Ile Thr Tyr Arg Ala Ala
359 180 185 190
362 Phe Gly Ser Ala Cys Glu Lys Gly Gln Asp Glu Phe Ile Arg Ile Leu
363 195 200 205
366 Gln Glu Phe Ser Lys Leu Phe Gly Ala Phe Asn Val Ala Asp Phe Ile
367 210 215 220
370 Pro Tyr Phe Gly Trp Ile Asp Pro Gln Gly Ile Asn Lys Arg Leu Val
371 225 230 235 240
374 Lys Ala Arg Asn Asp Leu Asp Gly Phe Ile Asp Asp Ile Ile Asp Glu
375 245 250 255
378 His Met Lys Lys Lys Glu Asn Gln Asn Ala Val Asp Asp Gly Asp Val
379 260 265 270
382 Val Asp Thr Asp Met Val Asp Asp Leu Leu Ala Phe Tyr Ser Glu Glu
383 275 280 285
386 Ala Lys Leu Val Ser Glu Thr Ala Asp Leu Gln Asn Ser Ile Lys Leu
387 290 295 300
390 Thr Arg Asp Asn Ile Lys Ala Ile Ile Met Asp Val Met Phe Gly Gly

```
VERIFICATION SUMMARY                              DATE: 03/22/2002
PATENT APPLICATION:  US/09/890,604                TIME: 11:40:37

Input Set : A:\41193--1.txt
Output Set: N:\CRF3\03222002\I890604.raw
```

```
L:12 M:271 C: Current Filing Date differs, Replaced Current Filing Date
```

#3

SEQUENCE LISTING

<110> Ellis, David D
Chapple, Clinton C S
Gilbert, Margarita

<120> Modification of Lignin Composition of Gymnosperms

<130> 41193-B

<140> 09/890,604
<141> 2001-08-01

<150> US 60/118,124
<151> 1999-02-01

<150> PCT/CA00/00074
<151> 2000-01-31

<160> 2

<170> PatentIn version 3.1

<210> 1
<211> 5156
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (2487)..(3002)
<223>

<220>
<221> CDS
<222> (3902)..(4519)
<223>

<220>
<221> CDS
<222> (3131)..(3556)
<223>

<400> 1
```
aagcttatgt atttccttat aaccatttta ttctgtatat agggggacag aaacataata      60

agtaacaaat agtggtttta ttttttttaaa tatacaaaaa ctgtttaacc attttatttc     120

ttggttagca aaattttgat atattcttaa gaaactaata ttttaggttg atatattgca     180

gtcactaaat agttttaaaa gacacgaagt tggtaagaac aggcatatat tattcgattt     240

aattaggaat gcttatgtta atctgattcg actaattaga aacgacgata ctatgagctc     300
```

atagatggtc ccacgaccca ctctcccatt tgatcaatat tcaactgagc aatgaaacta 360

attaaaaacg tggttagatt aaaaaaataa attgtgcagg tagcggatat ataatactag 420

taggggttaa aaataaaata aaacaccaca gtattaaatt tttgtttcaa aagtattatc 480

aatagttttt ttgcttcaaa aatatcacaa atttttgtat gaaatatttc tttaacgaaa 540

ataaattaaa taaaatttaa aatttatatt tggagttcta tttttaattt agagttttta 600

ttgttaccac atttttttgaa ttattctaat attaatttgt gatattatta caaaaagtaa 660

aaatatgata ttttagaata ctattatcga tatttgatat tattgacctt agctttgttt 720

gggtggagac atgtgattat cttattacct ttttattcca tgaaactaca gagttcgcca 780

ggtaccatac atgcacacac cctcgtgaag ccgtgactta atatgatcta gaacttaaat 840

agtactacta attgtgtcat ttgaactttc tcctatgtcg gtttcacttc atgtatcgca 900

gaacaggtgg aatacagtgt ccttgagttt cacccaaatc ggtccaattt tgtgatatat 960

attgcgatac agacatacag cctacagagt tttgtcttag cccactggtt ggcaaacgaa 1020

attgtcttta tttttttatg ttttgttgtc aatgtgtctt tgtttttaac tagattgagg 1080

tttaatttta atacatttgt tagtttacag attatgcagt gtaatctgat aatgtaagtt 1140

gaactgcgtt ggtcaaagtc ttgtgtaacg cactgtatct aaattgtgag taacgacaaa 1200

ataattaaaa ttaaaggacc ttcaagtatt attagtatct ctgtctaaga tgcacaggta 1260

ttcagtaata gtaataaata attacttgta taattaatat ctaattagta aaccttgtgt 1320

ctaaacctaa atgagcataa atccaaaagc aaaaatctaa acctaactga aaaagtcatt 1380

acgaaaaaaa gaaaaaaaaa agagaaaaaa ctacctgaaa agtcatgcac aacgttcatc 1440

ttggctaaat ttatttagtt tattaaatac aaaaatggcg agtttctgga gtttgttgaa 1500

aatatatttg tttagccact ttagaatttc ttgttttaat ttgttattaa gatatatcga 1560

gataatgcgt ttatatcacc aatatttttg ccaaactagt cctatacagt catttttcaa 1620

cagctatgtt cactaattta aaacccactg aaagtcaatc atgattcgtc atatttatat 1680

gctcgaattc agtaaaatcc gtttggtata ctatttattt cgtataagta tgtaattcca 1740

ctagatttcc ttaaactaaa ttatatattt acataattgt tttctttaaa agtctacaac 1800

agttattaag ttataggaaa ttatttcttt tatttttttt tttttttagg aaattatttc 1860

ttttgcaaca catttgtcgt ttgcaaactt ttaaaagaaa ataaatgatt gttataattg 1920

attacatttc agtttatgac agattttttt tatctaacct ttaatgtttg tttccctgtt 1980

tttaggaaaa tcataccaaa atatatttgt gatcacagta aatcacggaa tagttatgac 2040

caagattttc aaagtaatac ttagaatcct attaaataaa cgaaatttta ggaagaaata 2100

atcaagattt taggaaacga tttgagcaag gatttagaag atttgaatct ttaattaaat 2160

attttcattc ctaaataatt aatgctagtg gcataatatt gtaaataagt tcaagtacat 2220

gattaatttg ttaaaatggt tgaaaaatat atatatgtag attttttcaa aaggtatact 2280

aattattttc atattttcaa gaaaatataa gaaatggtgt gtacatatat ggatgaagaa 2340

atttaagtag ataatacaaa aatgtcaaaa aaagggacca cacaatttga ttataaaacc 2400

tacctctcta atcacatccc aaaatggaga actttgcctc ctgacaacat ttcagaaaat 2460

aatcgaatcc aaaaaaaaca ctcaat atg gag tct tct ata tca caa aca cta 2513
Met Glu Ser Ser Ile Ser Gln Thr Leu
1 5

agc aaa cta tca gat ccc acg acg tct ctt gtc atc gtt gtc tct ctt 2561
Ser Lys Leu Ser Asp Pro Thr Thr Ser Leu Val Ile Val Val Ser Leu
10 15 20 25

ttc atc ttc atc agc ttc atc aca cgg cgg cga agg cct cca tat cct 2609
Phe Ile Phe Ile Ser Phe Ile Thr Arg Arg Arg Arg Pro Pro Tyr Pro
30 35 40

ccc ggt cca cga ggt tgg ccc atc ata ggc aac atg tta atg atg gac 2657
Pro Gly Pro Arg Gly Trp Pro Ile Ile Gly Asn Met Leu Met Met Asp
45 50 55

caa ctc acc cac cgt ggt tta gcc aat tta gct aaa aag tat ggc gga 2705
Gln Leu Thr His Arg Gly Leu Ala Asn Leu Ala Lys Lys Tyr Gly Gly
60 65 70

ttg tgc cat ctc cgc atg gga ttc ctc cat atg tac gct gtc tca tca 2753
Leu Cys His Leu Arg Met Gly Phe Leu His Met Tyr Ala Val Ser Ser
75 80 85

ccc gag gtg gct cga caa gtc ctt caa gtc caa gac agc gtc ttc tcg 2801
Pro Glu Val Ala Arg Gln Val Leu Gln Val Gln Asp Ser Val Phe Ser
90 95 100 105

aac cgg cct gca act ata gct ata agc tat ctg act tac gac cga gcg 2849
Asn Arg Pro Ala Thr Ile Ala Ile Ser Tyr Leu Thr Tyr Asp Arg Ala
110 115 120

gac atg gct ttc gct cac tac gga ccg ttt tgg aga cag atg aga aaa 2897
Asp Met Ala Phe Ala His Tyr Gly Pro Phe Trp Arg Gln Met Arg Lys
125 130 135

gtg tgt gtc atg aag gtg ttt agc cgt aaa aga gct gag tca tgg gct 2945
Val Cys Val Met Lys Val Phe Ser Arg Lys Arg Ala Glu Ser Trp Ala
140 145 150

tca gtt cgt gat gaa gtg gac aaa atg gtc cgg tcg gtc tct tgt aac 2993
Ser Val Arg Asp Glu Val Asp Lys Met Val Arg Ser Val Ser Cys Asn

155 160 165

gtt ggt aag ctacttcaca tattcaccac tcttgctata tatatgtgca 3042
Val Gly Lys
170

attaaacaaa tatgtaaaaa gtgaaagtac tcatttcttc tttctttagt atgtacttta 3102

acatttaacc aaaacaattg taggtaag cct ata aac gtc ggg gag caa att 3154
Pro Ile Asn Val Gly Glu Gln Ile
175 180

ttt gca ctg acc cgc aac ata act tac cgg gca gcg ttt ggg tca gcc 3202
Phe Ala Leu Thr Arg Asn Ile Thr Tyr Arg Ala Ala Phe Gly Ser Ala
185 190 195

tgc gag aag gga caa gac gag ttc ata aga atc tta caa gag ttc tct 3250
Cys Glu Lys Gly Gln Asp Glu Phe Ile Arg Ile Leu Gln Glu Phe Ser
200 205 210

aag ctt ttt gga gcc ttc aac gta gcg gat ttc ata cca tat ttc ggg 3298
Lys Leu Phe Gly Ala Phe Asn Val Ala Asp Phe Ile Pro Tyr Phe Gly
215 220 225

tgg atc gat ccg caa ggg ata aac aag cgg ctc gtg aag gcc cgt aat 3346
Trp Ile Asp Pro Gln Gly Ile Asn Lys Arg Leu Val Lys Ala Arg Asn
230 235 240

gat cta gac gga ttt att gac gat att atc gat gaa cat atg aag aag 3394
Asp Leu Asp Gly Phe Ile Asp Asp Ile Ile Asp Glu His Met Lys Lys
245 250 255 260

aag gag aat caa aac gct gtg gat gat ggg gat gtt gtc gat acc gat 3442
Lys Glu Asn Gln Asn Ala Val Asp Asp Gly Asp Val Val Asp Thr Asp
265 270 275

atg gtt gat gat ctt ctt gct ttt tac agt gaa gag gcc aaa tta gtc 3490
Met Val Asp Asp Leu Leu Ala Phe Tyr Ser Glu Glu Ala Lys Leu Val
280 285 290

agt gag aca gcg gat ctt caa aat tcc atc aaa ctt acc cgt gac aat 3538
Ser Glu Thr Ala Asp Leu Gln Asn Ser Ile Lys Leu Thr Arg Asp Asn
295 300 305

atc aaa gca atc atc atg gtaattatat ttcaaaaagc actagtcata 3586
Ile Lys Ala Ile Ile Met
310

gtcatgtttc ttaatgcgtt acgtaataat acttatccat tgaccagtta ttttctccta 3646

agttttttg tttgaattag gaaggtaatt ttctatttta ctagagaaag caacagattt 3706

tagcatgatc tttttttaat atatatagaa gcattgaata ttcagatcta caataattat 3766

gaaactaatg aagagacaaa aaatggagag agaaaaaaga aagagtggac tagtgtggat 3826

atatttaatt ctaatttgat tttattagga cgttatattt aattctaatt tgattttttt 3886

```
atttgatttt attag gac gtt atg ttt gga gga acg gaa acg gta gcg tcg     3937
                 Asp Val Met Phe Gly Gly Thr Glu Thr Val Ala Ser
                 315                 320                 325

gcg ata gag tgg gcc tta acg gag tta tta cgg agc ccc gag gat cta     3985
Ala Ile Glu Trp Ala Leu Thr Glu Leu Leu Arg Ser Pro Glu Asp Leu
            330                 335                 340

aaa cgg gtc caa caa gaa ctc gcc gaa gtc gtt gga ctt gac aga cga     4033
Lys Arg Val Gln Gln Glu Leu Ala Glu Val Val Gly Leu Asp Arg Arg
        345                 350                 355

gtt gaa gaa tcc gac atc gag aag ttg act tat ctc aaa tgc aca ctc     4081
Val Glu Glu Ser Asp Ile Glu Lys Leu Thr Tyr Leu Lys Cys Thr Leu
    360                 365                 370

aaa gaa acc cta agg atg cac cca ccg atc cct ctc ctc ctc cac gaa     4129
Lys Glu Thr Leu Arg Met His Pro Pro Ile Pro Leu Leu Leu His Glu
375                 380                 385                 390

acc gcg gag gac act agt atc gac ggt ttc ttc att ccc aag aaa tct     4177
Thr Ala Glu Asp Thr Ser Ile Asp Gly Phe Phe Ile Pro Lys Lys Ser
                395                 400                 405

cgt gtg atg atc aac gcg ttt gcc ata gga cgc gac cca acc tct tgg     4225
Arg Val Met Ile Asn Ala Phe Ala Ile Gly Arg Asp Pro Thr Ser Trp
            410                 415                 420

act gac ccg gac acg ttt aga cca tcg agg ttt ttg gaa ccg ggc gta     4273
Thr Asp Pro Asp Thr Phe Arg Pro Ser Arg Phe Leu Glu Pro Gly Val
        425                 430                 435

ccg gat ttc aaa ggg agc aat ttc gag ttt ata ccg ttc ggg tcg ggt     4321
Pro Asp Phe Lys Gly Ser Asn Phe Glu Phe Ile Pro Phe Gly Ser Gly
    440                 445                 450

cgt aga tcg tgc ccg ggt atg caa cta ggg tta tac gcg ctt gac tta     4369
Arg Arg Ser Cys Pro Gly Met Gln Leu Gly Leu Tyr Ala Leu Asp Leu
455                 460                 465                 470

gcc gtg gct cat ata tta cat tgc ttc acg tgg aaa tta cct gat ggg     4417
Ala Val Ala His Ile Leu His Cys Phe Thr Trp Lys Leu Pro Asp Gly
                475                 480                 485

atg aaa cca agt gag ctc gac atg aat gat gtg ttt ggt ctc acg gct     4465
Met Lys Pro Ser Glu Leu Asp Met Asn Asp Val Phe Gly Leu Thr Ala
            490                 495                 500

cct aaa gcc acg cgg ctt ttc gcc gtg cca acc acg cgc ctc atc tgt     4513
Pro Lys Ala Thr Arg Leu Phe Ala Val Pro Thr Thr Arg Leu Ile Cys
        505                 510                 515

gct ctt taagtttatg gttcgagtca cgtggcaggg ggtttggtat ggtgaaaact       4569
Ala Leu
    520
```

gaaaagtttg aagttgccct catcgaggat ttgtggatgt catatgtatg tatgtgtata 4629

cacgtgtgtt ctgatgaaaa cagatttggc tctttgtttg ccctttttttt tttttctttt 4689

aatggggatt ttccttgaat gaaatgtaac agtaaaaata agatttttttt caataagtaa 4749

tttagcatgt tgcaaagatc gatcttggat gagaacttct acttaaaaaa aaaaaaaaaa 4809

ttttttttta gttatttcac ctttttcttt tgttctggtt gtatggttgc cattgtgtca 4869

attaggggct ggaagttcgc tggttaaggc taaatcagag ttaaagttat aattttacaa 4929

gcccaacaaa aggtcgcaga ttaaaaccac atgatattta taaaaaaaat tctaaggttt 4989

ttattagttt tattttcagt ttactgagta ctatttactt ttttattttt tgcaaataaa 5049

tgtattttat catatttatg tttttgtta taaactccaa acatacaggt ttcattacct 5109

aaaaaaagac agagtggttt cgttaatttt gtttcattaa tctcgag 5156

<210> 2
<211> 520
<212> PRT
<213> Arabidopsis thaliana

<400> 2

Met Glu Ser Ser Ile Ser Gln Thr Leu Ser Lys Leu Ser Asp Pro Thr
1 5 10 15

Thr Ser Leu Val Ile Val Val Ser Leu Phe Ile Phe Ile Ser Phe Ile
20 25 30

Thr Arg Arg Arg Arg Pro Pro Tyr Pro Pro Gly Pro Arg Gly Trp Pro
35 40 45

Ile Ile Gly Asn Met Leu Met Met Asp Gln Leu Thr His Arg Gly Leu
50 55 60

Ala Asn Leu Ala Lys Lys Tyr Gly Gly Leu Cys His Leu Arg Met Gly
65 70 75 80

Phe Leu His Met Tyr Ala Val Ser Ser Pro Glu Val Ala Arg Gln Val
85 90 95

Leu Gln Val Gln Asp Ser Val Phe Ser Asn Arg Pro Ala Thr Ile Ala
100 105 110

Ile Ser Tyr Leu Thr Tyr Asp Arg Ala Asp Met Ala Phe Ala His Tyr

115 120 125

Gly Pro Phe Trp Arg Gln Met Arg Lys Val Cys Val Met Lys Val Phe
130 135 140

Ser Arg Lys Arg Ala Glu Ser Trp Ala Ser Val Arg Asp Glu Val Asp
145 150 155 160

Lys Met Val Arg Ser Val Ser Cys Asn Val Gly Lys Pro Ile Asn Val
165 170 175

Gly Glu Gln Ile Phe Ala Leu Thr Arg Asn Ile Thr Tyr Arg Ala Ala
180 185 190

Phe Gly Ser Ala Cys Glu Lys Gly Gln Asp Glu Phe Ile Arg Ile Leu
195 200 205

Gln Glu Phe Ser Lys Leu Phe Gly Ala Phe Asn Val Ala Asp Phe Ile
210 215 220

Pro Tyr Phe Gly Trp Ile Asp Pro Gln Gly Ile Asn Lys Arg Leu Val
225 230 235 240

Lys Ala Arg Asn Asp Leu Asp Gly Phe Ile Asp Asp Ile Ile Asp Glu
245 250 255

His Met Lys Lys Lys Glu Asn Gln Asn Ala Val Asp Asp Gly Asp Val
260 265 270

Val Asp Thr Asp Met Val Asp Asp Leu Leu Ala Phe Tyr Ser Glu Glu
275 280 285

Ala Lys Leu Val Ser Glu Thr Ala Asp Leu Gln Asn Ser Ile Lys Leu
290 295 300

Thr Arg Asp Asn Ile Lys Ala Ile Ile Met Asp Val Met Phe Gly Gly
305 310 315 320

Thr Glu Thr Val Ala Ser Ala Ile Glu Trp Ala Leu Thr Glu Leu Leu
325 330 335

Arg Ser Pro Glu Asp Leu Lys Arg Val Gln Gln Glu Leu Ala Glu Val
340 345 350

Val Gly Leu Asp Arg Arg Val Glu Glu Ser Asp Ile Glu Lys Leu Thr
        355   360   365

Tyr Leu Lys Cys Thr Leu Lys Glu Thr Leu Arg Met His Pro Pro Ile
    370   375   380

Pro Leu Leu Leu His Glu Thr Ala Glu Asp Thr Ser Ile Asp Gly Phe
385   390   395   400

Phe Ile Pro Lys Lys Ser Arg Val Met Ile Asn Ala Phe Ala Ile Gly
                405   410   415

Arg Asp Pro Thr Ser Trp Thr Asp Pro Asp Thr Phe Arg Pro Ser Arg
            420   425   430

Phe Leu Glu Pro Gly Val Pro Asp Phe Lys Gly Ser Asn Phe Glu Phe
        435   440   445

Ile Pro Phe Gly Ser Gly Arg Arg Ser Cys Pro Gly Met Gln Leu Gly
    450   455   460

Leu Tyr Ala Leu Asp Leu Ala Val Ala His Ile Leu His Cys Phe Thr
465   470   475   480

Trp Lys Leu Pro Asp Gly Met Lys Pro Ser Glu Leu Asp Met Asn Asp
                485   490   495

Val Phe Gly Leu Thr Ala Pro Lys Ala Thr Arg Leu Phe Ala Val Pro
            500   505   510

Thr Thr Arg Leu Ile Cys Ala Leu
        515   520

MODIFICATION OF LIGNIN COMPOSITION OF GYMNOSPERMS

TECHNICAL FIELD

This invention relates to the modification of the lignin composition of gymnosperm species, particularly conifer trees, to make such species more suitable for commercial exploitation.

BACKGROUND ART

Lignin is a cell wall component present in vascular plants that decreases the permeability of cells, contributes to the strength and rigidity of the stem, and protects microfibrils from chemical, physical, and biological attack (Bugos et al. 1991 [4]). [*Note: for full details of references mentioned herein, see the section below headed REFERENCES, the numbers provided in square brackets corresponding to the numbers in that section.*] Despite its advantage to the plant, lignin greatly affects the agro-industrial uses of plants. Lignin content and composition alter the digestibility and dietary conversion of herbaceous crops and are undesirable in the conversion of wood into paper and pulp (Campbell and Sederoff 1996 [6]). Although lignin can contribute up to 25% of the mass of wood, from a pulp and paper viewpoint, lignin does not contribute to the usable biomass in pulping and hence is waste. More importantly, the extraction of lignin during chemical pulping is a costly and difficult process, involving chemical removal. There is a negative correlation between the amount of lignin removed and fiber yield with chemical pulping. Therefore, because the removal of lignin from fibers is a major cost, the modification of both lignin content and composition is a major focus of several research establishments world wide. Of importance is that trees with altered lignin, either decreased content or modified composition to reduce the energy needed to extract the lignin, could allocate more resources to the production of pulpable biomass with decreased costs.

Chemically, lignin is a highly complex network of phenylpropanoid units derived from the oxidative polymerization of one or more of three monolignol precursors which are the end products of the three major branches of the phenylpropanoid pathway (as shown in Figure 1 of the accompanying drawings, introduced the section below headed BRIEF DESCRIPTION OF THE DRAWINGS). As shown in the figure, branch 1 of the pathway yields the monolignol ρ-coumaryl alcohol which makes up the ρ-hydroxyphenyl residue when polymerized into lignin and is present in both angiosperms and gymnosperms. Branch 2 yields the monolignol coniferyl alcohol which makes up the guaiacyl residues when polymerized into lignin and is present in both angiosperms and gymnosperms, yet is the predominant monolignol in gymnosperms. Branch 3 yields sinapyl alcohol which makes up the syringyl residues when polymerized into lignin and is present only in angiosperms, with very few exceptions. These exceptions include reports of syringyl lignin in the gymnosperm species *Podocarpus* and in some species of the *Gnetales*. However, these exceptions are considered rare and are usually not even mentioned in reviews on lignin biosynthesis.

The presence of syringyl residues in angiosperm lignin via branch 3 in the phenylpropanoid pathway accounts for angiosperm lignin being easier to remove during pulping than gymnosperm lignin. One reason syringyl-lignin is easier to remove during pulping, as compared to guaiacyl-lignin produced by gymnosperms, is that the C-5 carbon of the phenyl ring in syringyl-lignin is protected by methoxylation from forming a C5-C5 bond with adjacent monolignol phenyl rings. Once formed, this C-C bond is very difficult to break during delignification and the presence of these bonds accounts for the fact that gymnosperm lignin is harder to pulp than angiosperm lignin.

The inventors of the present invention theorized that if the phenylpropanoid pathway in gymnosperms could be modified such that gymnosperm plants could produce lignin containing syringyl residues, via branch 3, or a modification thereof, of the phenylpropanoid pathway, this would be of great benefit because significant reductions in the pulping costs associated with lignin removal in gymnosperms would be enabled.

However, this requires the creation of an entirely new pathway in gymnosperms, i.e., the creation of the enzymes and substrates in gymnosperm species to enable the branch 3 phenylpropanoid pathway synthesis of syringyl-lignin to proceed through to completion. This is quite different in concept from arranging for over-expression of a gene in an existing metabolic pathway, which is likely to shuttle more metabolites through the pathway, provided other steps do not become limiting.

There are numerous reports on the modification of the phenylpropanoid pathway by genetic engineering. One example is the “sense” suppression of PAL by a bean PAL2 gene in tobacco. These experiments demonstrated that PAL activity becomes rate-limiting to lignin deposition when levels are 3- to 4-fold lower than in wild-type plants (Bate et al. 1994 [2]). While PAL may hold promise for use in engineered lignin modification, it has been suggested that due to its key role in general phenylpropanoid metabolism, the interruption of PAL synthesis would also affect other biochemical pathways. In contrast, the activity of CAD, an enzyme well downstream in the lignin biosynthetic pathway, can be reduced to 10% of normal levels and still have no effect on the quantity of lignin, although clear qualitative differences are observed (Halpin et al. 1994 [12]). From these and other studies on the manipulation by genetic engineering of key enzymes in the lignin biosynthetic pathway (OMT (Dwivedi et al. 1994 [10]; Ni et al. 1994 [16]), F5H (Bell-Lelong et al. 1997, [3]), and peroxidase (Lagrimini et al. 1990

[14])), it is clear that lignin modification is possible. However, such studies also highlight how extremely difficult it is to achieve a change in lignin composition and how it is even more difficult to achieve a change that has commercial relevance. In the CAD antisense work, Halpin et al. [12] reported increased lignin extractability in only 2% of the transformed lines tested. In other words, 98% had no change despite morphological changes such as the appearance of red xylem.

Therefore, these disclosures do not specifically relate to techniques involving genetic engineering to create a lignin which is unique to the plants of interest, i.e. gymnosperms. Firstly, all the published work on the genetic engineering of plants for altered lignin has been done in angiosperms and was done to manipulate an existing endogenous enzyme and biochemical pathway. Even with this, the results were variable, and changing lignin parameters to a level such that they had commercial advantages was difficult. Secondly, the only example of lignin modification in gymnosperms where a gene for a specific enzyme in the phenylpropanoid pathway was down-regulated occurred in a naturally occurring mutant which had virtually no CAD activity (for a review see Whetton et al. 1998 [17]). In this case, genetic engineering was not used and the regulation was again dependent on natural mutation which altered the expression of an endogenous gene.

International patent application PCT/US96/20094, published on July 3, 1997 as WO 97/23599, in the name of Clint Chapple as inventor, and assigned jointly to E.I. Du Pont De Nemours and Company, and Purdue Research Foundation, discloses the nucleotide sequence of a gene encoding an F5H enzyme, the transformation of the genome of plants with the gene, and the resulting modification of lignin composition of the plants. The present application builds on this Chapple application and goes beyond, to describe

the use of this gene, either alone or in conjunction with other genes, to introduce a lignin biosynthetic pathway into gymnosperms.

DISCLOSURE OF THE INVENTION

An object of the invention is to modify gymnosperms by genetic engineering so that modified gymnosperm plants produce lignin of a type that differs from the lignin of wild-type plants of the same species and that is more easily accommodated in commercial utilization of such plants.

Another object of the invention is to modify the lignin precursors in gymnosperms to provide modified monolignol residues, and preferably, a greater content of syringyl residues, or other residues with a side group at the C-5 position of the monolignol ring.

According to one aspect of the present invention, there is provided a process of producing a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in modification of lignin composition in the gymnosperm plant compared to an average lignin composition of untransformed wild-type plants of the same gymnosperm species, which process comprises: providing a vector containing at least one expressible transgene that results in modification of the lignin composition in the gymnosperm plant; introducing the vector into cells of a gymnosperm plant to produce transformed cells; regenerating transformed gymnosperm callus or shoots from the transformed cells; maturing embryos or plants from the transformed callus or shoots; and generating transformed plant embryos, seeds, seedlings or plants from the matured embryos.

Without wishing to limit the generality of meaning of the term "transgene", we should point out that the term is intended to include foreign DNA (transgenic or introduced genes) that is introduced into a genome of a gymnosperm plant.

According to another aspect of the invention, there is provided a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in modification of lignin composition in the gymnosperm plant compared to an average lignin composition of untransformed wild-type plants of the same gymnosperm species.

Most preferably, the lignin of the transformed gymnosperm plant contains detectable syringyl residues, or other residues with a side group at the C-5 position of the monolignol ring, whereas the lignin of the wild-type plants contains no detectable syringyl residues or other residues with a side group at the C-5 position of the monolignol ring.

Preferably, the expressible transgenes are genes that code for enzymes required for the lignin biosynthetic pathway, and more preferably the third branch of the pathway by which branch 2 intermediates are converted to sinapyl alcohol. It is therefore to be noted that, in the present invention, at least in its preferred forms, gymnosperm plants are being genetically engineered with genes which encode at least one enzyme that is not normally present in these plants, thereby creating a branch to an existing pathway in gymnosperm plants. The invention therefore differs considerably from prior art procedures that have merely involved the modification of existing pathways in angiosperm plants utililizing enzymes already present in the wild-type plants.

Most preferably, the transgene(s) introduced into the gymnosperm plants includes a ferulate 5-hydroxylase gene, or a transgene that is substantially homologous to said ferulate 5-hydroxylase gene, or a transgene that has an equivalent function, either alone or in conjunction with other genes needed for the biosynthesis of a lignin, i.e. that results in a lignin composition containing syringyl residues. By a "gene that is substantially homologous to said ferulate 5-hydroxylase gene", we mean a gene which can be shown to have ferulate 5-hydroxylase activity in yeast or having at least 50% homology, and more preferably at least 75% homology, to the F5H gene while exhibiting an ability to modify the lignin content of the gymnosperm plant *in vivo*.

The ferulate 5-hydroxylase gene (or equivalent gene) either alone or in conjunction with other genes, are normally operably linked with at least one regulatory sequence, e.g. cauliflower mosaic virus 35S promoter, a promoter for a phenylalanine ammonia lyase gene, a promoter for a p-coumaryl CoA ligase gene, a promoter for cinnamate 4-hydroxylase or other plant promoters capable of controlling expression of plant genes.

The gymnosperm plants produced by the present invention are preferably from the order *coniferales*. Thus, they may be from the *Picea* species (e.g. *Picea glauca, Picea sitchesis*, or *Picea engelmanii*), or from the *Pinus* species (e.g. *Pinus taeda* or *Pinus radiata*).

According to another aspect of the invention, there is provided a biomass derived from a genetically transformed gymnosperm plant, said biomass containing lignin having syringyl residues, or other residues with a side group at the C-5 position of the monolignol ring, and said transformed plant having an untransformed (wild-type) natural plant whose lignin contains no syringyl residues.

A still further aspect of the invention relates to a method of producing cellulose-containing pulp useful for papermaking and the like, which comprises a lignin-containing biomass derived from a gymnosperm plant to produce pulped mass containing lignin, and removing most of said lignin from said pulped mass, characterized in that said gymnosperm plant is a genetically transformed plant producing lignin containing syringyl residues or other residues with a side group at the C-5 position of the monolignol ring.

As will be appreciated from the above, the present invention is capable of producing transformed gymnosperm plants having a modified lignin content that makes gymnosperm plants more attractive on a commercial and industrial scale.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing the basic lignin biosynthetic pathway, the enzyme abbreviations being as described in this application, and the suggested induced pathway(s) being highlighted (the inserted box indicates the numbering in the phenyl ring). Note the three branches of the phenylpropanoid pathway (labeled 1), 2) and 3)): branch 1 yields the monolignol ρ-coumaryl alcohol, present in some angiosperms and gymnosperms; branch 2 yields the monolignol coniferyl alcohol, which is present in both angiosperms and gymnosperms yet is the predominant monolignol in gymnosperms; and branch 3 yields sinapyl alcohol predominant and present only in angiosperms, with very few exceptions.

Figure 2 is a graph showing the mean height growth in 1997 from three different transformed lines derived from two different parental genotypes of F5H-transformed and control (non-transformed) interior spruce somatic

seedlings (note F5H 2d-1 and 2d–2 are two replicate sets of somatic seedlings planted 2 weeks apart);

Figure 3 shows the result of a PCR amplification of a 750 bp arabidopsis F5H fragment using the primer pair cc8/cs278 from 14 Putative Transformed Lines (lanes 1-14); a Non-Transformed I1026 Negative Control (lane 15); a Plasmid only p482-F5H = pCC87Positive Control (lane 16); a Plasmid only pBIC-F5H = pBIC20F5H, Control (lane 17); a Blank, no DNA Negative Control (lane 18); and Molecular Weight (MW) Markers (lane 19); and

Figure 4 shows the genomic nucleotide and amino acid sequences of a known Arabidopsis F5H gene and the F5H enzyme that it encodes (as disclosed in Chapple, WO 97/23599).

BEST MODES FOR CARRYING OUT THE INVENTION

As previously noted, the difficulty in lignin removal in all plants is due to the variety of linkages formed between monolignol precursors during lignin polymerization, which linkages account for lignin polymers being highly heterogeneous. This heterogeneity in lignin and the linkages formed during polymerization have a large influence on the pulping characteristics of wood. For example, the presence of the C-5 methoxylated syringyl residues make hardwood lignin easier to hydrolyze during pulping, while a higher proportion of condensed ρ-hydroxyphenyl residues makes softwood hydrolysis more difficult (Campbell and Sederoff 1996 [6]). This is due in part to the unprotected C-5 group characteristic of ρ-hydroxyphenyl and guaiacyl residues typical of softwoods, accounting for the relatively slower delignification rate of the softwoods (Chiang and Funaoka 1990 [8]).

Any modification of this complex polymer requires an understanding of the metabolic pathway. Fortunately, many steps in the lignin biosynthetic pathway are well understood (Davin et al. 1992[9]). The basic phenylpropanoid pathway is shown in accompanying Figure 1. The pathway begins with the conversion of L-phenylalanine to cinnamic acid, by phenylalanine ammonia-lyase (PAL) followed by the conversion of cinnamate to 4-coumarate by cinnamate 4-hydroxylase (C4H). 4-Coumarate has several potential metabolic fates and these account for pathways to the three monolignol precursors. Thus, 4-coumarate can enter into one of the three monolignol branches of the phenylpropanoid pathway shown in Figure 1 as follows:

Branch 1) Present in both angiosperms and gymnosperms where 4-coumarate is activated to 4-coumaryl-CoA in a reaction catalyzed by 4-coumaryl-CoA ligase (4CL), and reduced by hydroxycinnamyl-CoA reductase (CCR) and cinnamyl alcohol: NAD oxidoreductase (CAD) to 4-hydroxycinnamyl alcohol (ρ -coumaryl alcohol), the first of the three monomeric lignin precursors;

Branch 2) Present in both angiosperms and gymnosperms where 4-coumerate is either: A) 3-hydroxylated and 3-O-methylated to form ferulic acid, followed by activation by 4CL, and reduced by CCR and CAD to yield 3-methoxy-4-hydroxycinnamyl alcohol (coniferyl alcohol), the major lignin precursor in conifers; or B) activated to 4-coumarly-CoA which is subsequently 3-hydroxylated and 3-O-methylated to form feruloyl-CoA, which is then reduced by both CCR and CAD to yield 3-methoxy-4-hydroxycinnamyl alcohol; and

Branch 3) Present only in angiosperms where 4-coumarate is modified as in branch 2); however, either: A) ferulic acid undergoes a ring-hydroxylation by ferulate 5-hydroxylase (F5H) and O-methylation by an O-methyltransferase (OMT) to generate sinapic acid, which is reduced to yield sinapyl alcohol, the source of the syringyl residues typical in angiosperms; or B) proceeds through branch 2 to coniferaldehyde and then to 5-hydroxyconiferaldehyde to sinapaldehyde to sinapyl alcohol; or C) proceeds through branch 2 to coniferaldehyde and then to 5-hydroxyconiferyl alcohol to sinapyl alcohol.

The present invention involves the genetic engineering of gymnosperms to introduce one or more functional genes encoding one or more enzymes that results in a modification of the lignin composition of a gymnosperm plant that makes the gymnosperm plant or plant products more commercially desirable. The modification of gymnosperms with genes for any of the enzymes capable of affecting the phenylpropanoid pathway is within the scope of the invention, provided such genes modify the lignin composition of gymnosperm plants to make the plants more commercially desirable. Preferably, the transgene creates a Branch 3 metabolic pathway, or other residues with a side group at the C-5 position of the monolignol ring, and most preferably one of the genes encodes ferulate 5-hydroxylase (F5H). As noted above, this enzyme is thought to be absent in most gymnosperms (with few exceptions) and is one of the key enzymes missing in conifers which accounts for the difference between angiosperm and gymnosperm lignin (Campbell and Sederoff 1996 [6]). The exceptions are very few as previously noted and include reports of syringyl lignin in the non-coniferales gymnosperm species *Podocarpus* and in some species of the *Gnetales*. These exceptions do not, however, detract from the invention as the vast

number of gymnosperms do not produce syringyl lignin and these exceptions are mentioned in a very minor way, if at all in the literature on the subject.

The inventors have been successful in expressing the F5H gene in spruce (a gymnosperm) and have transformed lines containing this transgene in conjunction with other transgenes in the lignin biosynthetic pathway. Since the inventors have demonstrated expression of the F5H gene in spruce, they believe that its expression in other gymnosperm species is predictable since this clearly shows that not only the F5H gene can be expressed in gymnosperms, but also that its expression can modify lignin in plants which do not contain a pathway for syringyl lignin.

Although not conclusive, support that this single enzyme (F5H), either alone or in conjunction with other enzymes, will alter gymnosperm lignin comes from the fact that mutants of the angiosperm *Arabidopsis* which lack this enzyme produce lignin similar in composition to gymnosperms (Chapple et al. 1992), suggesting the lack of this one enzyme, alone or in conjunction with other enzymes, can account for the difference in lignin composition in an angiosperm where a branch of the phenylpropanoid pathway to guaiacyl-containing lignin already exists.

As noted above, the F5H gene is known and described, e.g. in PCT publication WO 97/23599 on July 3, 1997. The disclosure of this publication is specifically incorporated herein by reference. For convenience, the nucleotide sequence of the F5H gene from *Arabidopsis* and the amino acid sequence of the F5H enzyme is shown in Figure 4 of the accompanying drawings.

The F5H gene can be obtained from an angiosperm species, e.g. *Arabidopsis thaliana,* DNA either by polymerase chain reaction (PCR) using

primers designed to the 5' and 3' ends of the published F5H sequence in Figure 4, or by plasmid rescue of the *fah*1 mutant and complementation as was done by Meyer et al. (1996[15]). The PCR amplified product can then be used to identify the native gene from either a genomic or cDNA library and the gene can be subsequently cloned by standard gene cloning techniques. The isolation of the gene by PCR or from the *fah*1 mutant is believed to be within the competence of any person skilled in the art, so that further explanation is unnecessary. Similar techniques can and have been used to isolate other genes in the lignin biosynthetic pathway which can be used in conjunction with an F5H gene to modify lignin in gymnosperms.

Several constructs of the F5H gene were obtained as explained in the PCT publication mentioned above. These constructs include either genomic and cDNA F5H genes controlled by a cauliflower mosaic virus (CaMV) 35S or cinnimate 4-hydroxylase (C4H) promoter, as well as a C4H-GUS construct to test the expression pattern of the C4H promoter, as well as an OMT construct used in conjunction with F5H and a construct containing an F5H-OMT translational fusion. These and other constructs used in this invention are listed below:

pGA482-F5H = pCC87	a pGA482-based vector containing a CaMV 35S-genomic F5H construct;
pBIC-F5H = pBIC20-F5H	a pBIC20-based vector containing an 18kb genomic fragment containing both the F5H promoter and coding region;
pCC98	a pBI121-based vector containing a CaMV 35S-cDNA F5H construct;

pCC223	a pBI101-based vector containing a C4H-GUS construct for xylem directed expression of GUS;
pC4H-F5H = pCC153	a pGA482-based vector containing a C4H-genomic F5H construct; and
pCC99	a pGA482-based vector containing a double-CaMV 35S-genomic F5H construct.
parabOMT	a pUC based vector containing a CaMV 35S-OMT construct used for co-blasting with pCC99.
pF5H-OMT	a pBINPLUS derived vector containing a double-CaMV 35S-F5H-OMT translational fusion.

Starting materials for such vectors are in common use for the construction of plant transformation vectors and are generally available around the world from various labs. The pBI- series is commercially available from Clontech. The pGA482 vector is described in 1987 Methods Enzymol 153:292-305 and is widely used for plant transformation. The pBIC20is a binary cosmid vector described by Meyer et al. 1996, in Genome Mapping in Plants, ed. Paterson, A.H. (Landis Biochemical Press, Austin, TX). Construction of the pGA482-F5H and pBIC20-F5H plasmids are detailed in Meyer et al., 1996, PNAS, 93.6869-6874 and both are available from that source (Chapple). The other F5H and OMT constructs were made using similar techniques.

As seen in the PCT publication mentioned above, the CaMV 35S constructs have been used successfully to modify lignin content in both *Arabidopsis* and tobacco and were included in the present invention to give ectopic expression of the F5H gene in spruce. The C4H promoter constructs should

direct expression to the xylem, the principal target tissue for lignin modification. Because the C4H promoter was isolated from an *Arabidopsis* C4H gene, its expression - as well as the expression of the native C4H gene - in gymnosperms was previously unknown. The OMT constructs were included to ensure, if needed, the O-methylation of the 5-hydroxylated branch 2 intermediates.

To initiate transformation experiments, the plasmids were transformed into *E. coli* and were subsequently purified by CsCl gradient centrifugation. Each plasmid was checked by restriction digest to confirm its identity. Standard procedures were used for coating gold particles with the plasmids and for microprojectile bombardment of spruce somatic embryos. Regeneration of transformed spruce callus was done on a very low level of kanamycin (2–5μg/ml) and embryo maturation was done using routine protocols for spruce.

Over 10,000 spruce embryos were blasted with the various constructs and over 500 transformed seedlings from over 50 transformed lines have been planted in the greenhouse. No abnormal phenotypes or altered growth patterns have been detected in any of the transformed embryogenic callus lines or seedlings. The results of these experiments are summarized in Table 1 below.

Table 1

Information regarding tested constructs

CONSTRUCT	# KANAMYCIN RESISTANT LINES	# CONFIRMED PCR POSITIVE LINES	# CONFIRMED SOUTHERN POSITIVE LINES	# CONFIRMED NORTHERN POSITIVE LINES	# OF LINES WITH SEEDLINGS REGENERATED
pCC87 35S-gF5H	16	16	8	5	18
pBIC20-F5H F5H-gF5H	6	Inconclusive	Inconclusive	Inconclusive	6
pCC98 35S-cF5H	5	2 – Inconclusive	2	nd	1
pCC223 C4H-GUS	6	5	3	ND	5
pCC153 C4H-gF5H	16	10	6	2	12
pCC99 2X 35S-gF5H	20	19	3	17	18
pCC99+ parabOMT	11	Nd	nd	nd	0
Total	80	47	22	24	60

Figure 2 of the accompanying drawings shows the mean height growth of three different F5H transformed lines from two different parental embryogenic genotypes compared with non-transformed somatic seedlings over the growing season. Transformed line I1026 2d is represented by two lots of somatic seedlings planted two weeks apart.

A set of six nested primers for the F5H gene were obtained and tested for amplification of the F5H gene from pCC87.The primer pair consisting of cc8 and cs278 were used to amplify a band of approximately 750bp from the genomic F5H gene.

Figure 3 confirms integration by PCR of the Arabidopsis F5H in 14 different putative F5H transformed embryogenic callus lines (lanes 1–14). A band of approximately 300bp in all lanes including the blank (lane 18) and the non-transformed I1026 control (lane 15), suggests that this fragment is a non-specific amplification product. The band of interest, a 750bp amplification product, is very prominent as expected in the pCC87 plasmid only lane (lane 16) and is absent from both the blank and the non-transformed control. Note the presence of a 750bp band in DNA samples from 10 transformed lines (lanes 1,3,6,7,8,9,11,12,13,14) including the three transformed lines which have somatic seedlings in the greenhouse. The absence of the 750bp band in the remaining putative transformed lines could indicate that these lines are non-transformed escapes, or that the DNA preparation from these lines was poor. This later suggestion is supported by the lack of other background bands in these lanes (lanes 2,4,5,10).

Northern blot analyses have confirmed strong expression of the F5H gene in spruce and Southern blot analysis of transformed lines have conclusively confirmed the PCR results for integration of the inserted DNA into the spruce genome (Table 1).

REFERENCES

1. Bao et al. 1993. Science 260: 672-676;
2. Bate et al. 1994. Proc Natl Acad Sci USA 84:980-984;
3. Bell-LeLong et al. 1997. Plant Physiol, Plant Physiol 113(3):729.
4. Bugos et al. 1991. Plant Mol Biol 17:1203-1215;
5. Campbell and Ellis. 1992. Planta 186:409-417;
6. Campbell and Sederoff. 1996. Plant Physiol 110:3-13;
7. Chapple et al. 1992. Plant Cell 4:1413-1424;
8. Chiang and Funaoka. 1990. Holzforschung 44:147-155.
9. Davin et al. 1992. Rec Adv Phytochem 26: 325-376;
10. Dwivedi et al. 1994. Plant Mol Biol 26:61-71;
11. Ellis et al. 1993. Bio/Technology 11:84-89;
12. Halpin et al. 1994. Plant J 6:339-350;
13. Iiyama and Wallis. 1990. J Sci Food Agric 51: 145-161;
14. Langrimini et al. 199 Plant Cell 2:7-8;
15. Meyer et al. 1996. PNAS 93:6869-6874;
16. Ni WT, Paiva NL and Dixon RA. 1994 Transgenic Res 3(2):120-126
17. Whetton, R.W., MacKay, J.J., and Sederoff, R.R. 1998 Ann. Rev. Plant Physiol. Plant Mol. Biol. 49:585-609.

The disclosures of all of the above publications are specifically incorporated herein by reference.

CLAIMS:

1. A process of producing a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in modification of lignin composition in the gymnosperm plant compared to an average lignin composition of untransformed wild type plants of the same gymnosperm species, which process comprises:

providing a vector containing at least one expressible transgene that results in modification of the lignin composition in the gymnosperm plant;

introducing said vector into cells of a gymnosperm to produce transformed cells;

regenerating transformed gymnosperm callus or shoots from the transformed cells;

maturing embryos from the transformed callus or shoots; and

generating transformed plant embryos, seeds, seedlings or plants from the matured embryos or shoots.

2. A process according to claim 1, characterized in that said vector is provided with said at least one expressible transgene that encodes at least one enzyme affecting the phenylpropanoid pathway leading to the synthesis of lignin.

3. A process according to claim 1, characterized in that said vector is provided with said expressible transgene that encodes at least one enzyme enabling the production of sinapyl alcohol or other residues with a side group at the C-5 position of a monolignol ring during the biosynthesis of lignin.

4. A process according to claim 1, characterized in that said vector is provided with said at least one expressible transgene that encodes at least one enzyme enabling the production of lignin containing syringyl residues or other residues with a side group at the C-5 position of a monolignol ring.

5. A process according to claim 4, characterized in that said vector is provided with an expressible transgene encoding ferulate 5-hydroxylase, or a transgene that has substantially equivalent function to said ferulate 5-hydroxylase gene, either alone or in conjunction with other genes involved in lignin biosynthesis.

6. A process according to claim 5, characterized in that one of the said substantially homologous gene has at least 50% homology with said ferulate 5-hydroxylase gene.

7. A process according to claim 5, characterized in that said substantially homologous gene has at least 75% homology with said ferulate 5-hydroxylase gene.

8. A process according to any preceding claim, characterized in that said gymnosperm plant is from the order coniferales.

9. A process according to any preceding claim, characterized in that said gymnosperm plant is from the species *Picea*.

10. A process according to claim 9, characterized in that said plant is *Picea glauca, Picea sitchesis*, or *Picea engelmanii.*

11. A process according to any one of claims 1 to 8, characterized in that said gymnosperm plant is from the species *Pinus*.

12. A process according to claim 11, characterized in that said gymnosperm plant is *Pinus taeda* or *Pinus radiata*.

13. A process according to claim 5, characterized in that said ferulate 5-hydroxylase gene is operably linked with at least one regulatory sequence.

14. A process according to claim 13, characterized in that said regulatory sequence is cauliflower mosaic virus 35S promoter, a promoter for a phenylalanine ammonia lyase gene, a promoter for a p-coumaryl CoA ligase gene, a promoter for cinnamate 4-hydroxylase, or another plant promoter capable of controlling expression of plant genes.

15. A transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in modification of lignin composition in the gymnosperm plant compared to an average lignin composition of untransformed wild type plants of the same gymnosperm species.

16. A gymnosperm plant or plant precursor according to claim 15, characterized in that said plant has a genome containing at least one expressible transgene that encodes at least one enzyme enabling the production of sinapyl alcohol, or other residue with a side group at the C-5 position of a monolignol ring, during the biosynthesis of lignin.

17. A gymnosperm plant or precursor according to claim 15, characterized in that the plant or plant precursor has a genome containing an

expressible transgene that results in a lignin composition containing syringyl residues, or other residue with a side group at the C-5 position of a monolignol ring.

18. A gymnosperm plant or precursor according to claim 15, characterized in that said at least one expressible transgene is a gene encoding ferulate 5-hydroxylase, or a transgene that has substantially equivalent function to said ferulate 5-hydroxylase gene, either alone or in conjunction with other genes involved in lignin biosynthesis.

19. A gymnosperm plant or precursor according to claim 18, characterized in that said substantially homologous gene has at least 50% homology with said ferulate 5-hydroxylase gene.

20. A gymnosperm plant or precursor according to claim 18, characterized in that said substantially homologous gene has at least 75% homology with said ferulate 5-hydroxylase gene.

21. A gymnosperm plant or precursor according to claim 15, characterized in that said gymnosperm plant is from the order coniferales.

22. A gymnosperm plant or precursor according to claim 15, characterized in that said gymnosperm plant is from the species *Picea*.

23. A gymnosperm plant or precursor according to claim 22, characterized in that said plant is *Picea glauca, Picea sitchesis*, or *Picea engelmanii*.

24. A gymnosperm plant or precursor according to claim 15, characterized in that said gymnosperm plant is from the species *Pinus*.

25. A gymnosperm plant or precursor according to claim 24, characterized in that said gymnosperm plant is *Pinus taeda* or *Pinus radiata*.

26. A gymnosperm plant or precursor according to claim 18, characterized in that said ferulate 5-hydroxylase gene is operably linked with at least one regulatory sequence.

27. A gymnosperm plant or precursor according to claim 26, characterized in that said regulatory sequence is a cauliflower mosaic virus 35S promoter, a promoter for a phenylalanine ammonia lyase gene, a promoter for a p-coumaryl CoA ligase gene, a promoter for cinnamate 4-hydroxylase, or any other plant promoter capable of controlling expression of plant genes.

28. A biomass derived from a genetically transformed gymnosperm plant, said biomass containing lignin having syringyl residues, or other residue with a side group at the C-5 position of a monolignol ring, and said transformed plant having an untransformed natural wild-type plant whose lignin contains no syringyl residues, or corresponding other residues with a side group at the C-5 position of a monolignol ring.

29. A biomass according to claim 28, resulting from growing and harvesting a genetically transformed plant or plant precursor as defined in any one of claims 15 to 27.

30. A method of producing a cellulose-containing pulp useful for papermaking and the like, which comprises finely dividing a lignin-containing biomass derived from a gymnosperm plant to produce pulped mass containing lignin, and removing at least some of said lignin from said pulped mass, characterized in that said gymnosperm

plant is a genetically transformed plant producing lignin containing syringyl residues or other residues with a side group at the C-5 position of a monolignol ring.

31. A process of producing a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in production of at least one residue of a lignin biosynthetic pathway having a hydroxy group at the C-5 position of a monolignol ring, which process comprises:

providing a vector containing at least one expressible transgene that results in production of at least one residue having a hydroxy group at the C-5 position of a monolignol ring;

introducing said vector into cells of a gymnosperm to produce transformed cells;

regenerating transformed gymnosperm callus or shoots from the transformed cells;

maturing embryos from the transformed callus or shoots; and

generating transformed plant embryos, seeds, seedlings or plants from the matured embryos or shoots.

32. A process of producing a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene encoding an enzyme enabling hydroxylation at the C-5 position of a monolignol ring of at least one residue in a lignin biosynthetic pathway, which process comprises:

providing a vector containing at least one expressible transgene encoding an enzyme enabling hydroxylation at the C-5 position of a monolignol ring of at least one residue;

introducing said vector into cells of a gymnosperm to produce transformed cells;

regenerating transformed gymnosperm callus or shoots from the transformed cells;

maturing embryos from the transformed callus or shoots; and

generating transformed plant embryos, seeds, seedlings or plants from the matured embryos or shoots.

33. The process of claim 32, wherein the enzyme is selected from the group comprising ferulate-5-hydroxylase and homologs thereof.

34. A process of producing a transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in hydroxylation of at least one residue of a lignin biosynthetic pathway at the C-5 position of a monolignol ring, which process comprises:

providing a vector containing at least one expressible transgene that results in hydroxylation of at least one residue at the C-5 position of a monolignol ring;

introducing said vector into cells of a gymnosperm to produce transformed cells;

regenerating transformed gymnosperm callus or shoots from the transformed cells;

maturing embryos from the transformed callus or shoots; and

generating transformed plant embryos, seeds, seedlings or plants from the matured embryos or shoots.

35. A transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that results in hydroxylation of at least one residue of a lignin biosynthetic pathway at the C-5 position of a monolignol ring.

36. A transformed gymnosperm plant or plant precursor having a genome containing at least one expressible transgene that encodes at least one enzyme enabling the production of a residue of a lignin biosynthetic pathway with a side group at the C-5 position of a monolignol ring, during the biosynthesis of lignin.

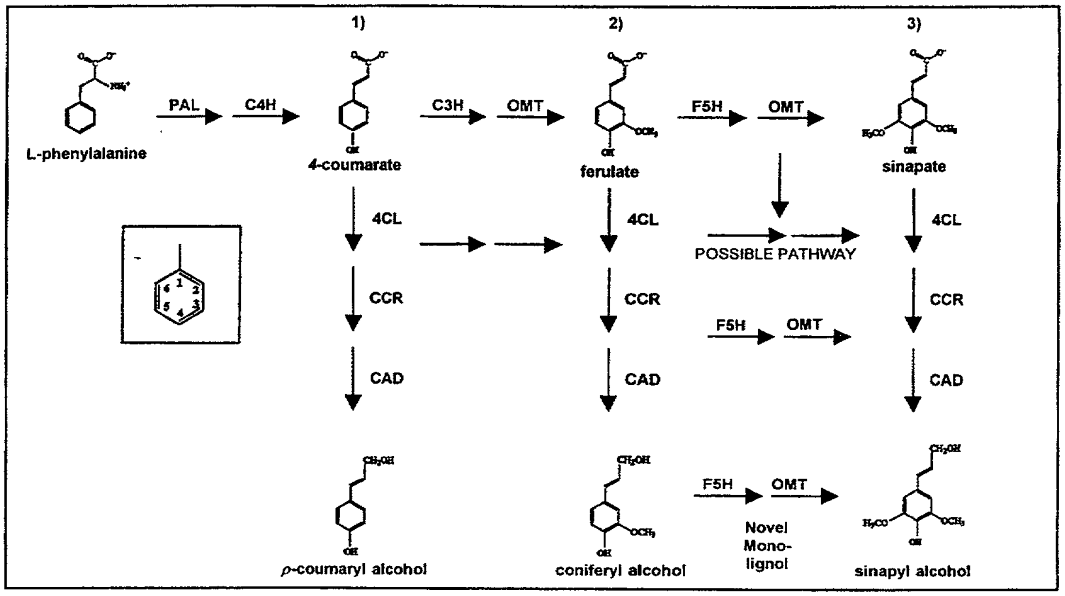
1)
2)
3)
PAL
C4H
C3H
OMT
F5H
OMT
L-phenylalanine
4-coumarate
ferulate
sinapate
4CL
CCR
CAD
POSSIBLE PATHWAY
F5H
OMT
p-coumaryl alcohol
coniferyl alcohol
Novel Mono-lignol
sinapyl alcohol

Fig. 1

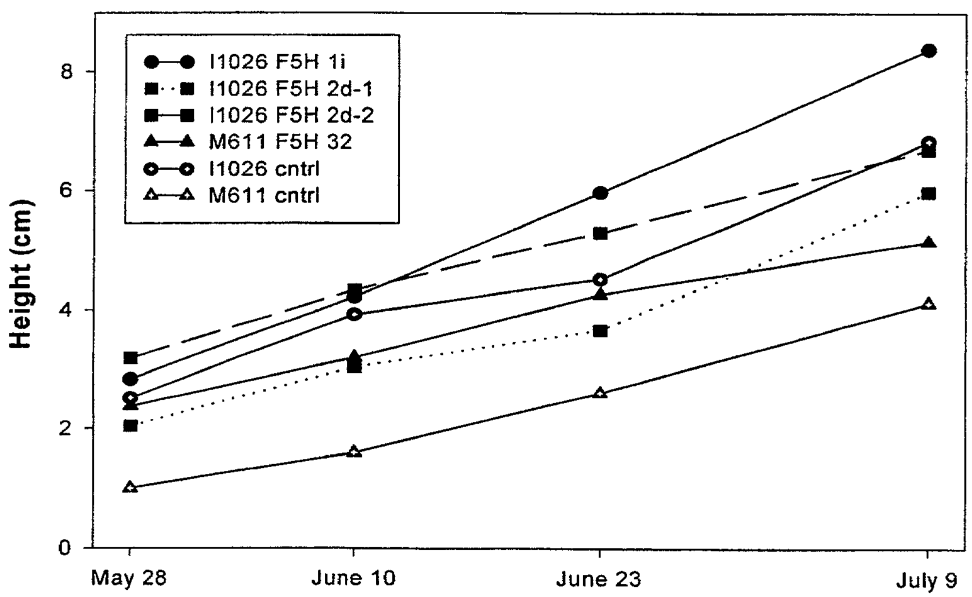
I1026 F5H 1i
I1026 F5H 2d-1
I1026 F5H 2d-2
M611 F5H 32
I1026 cntrl
M611 cntrl
Height (cm)
0
2
4
6
8
May 28
June 10
June 23
July 9

Fig. 2

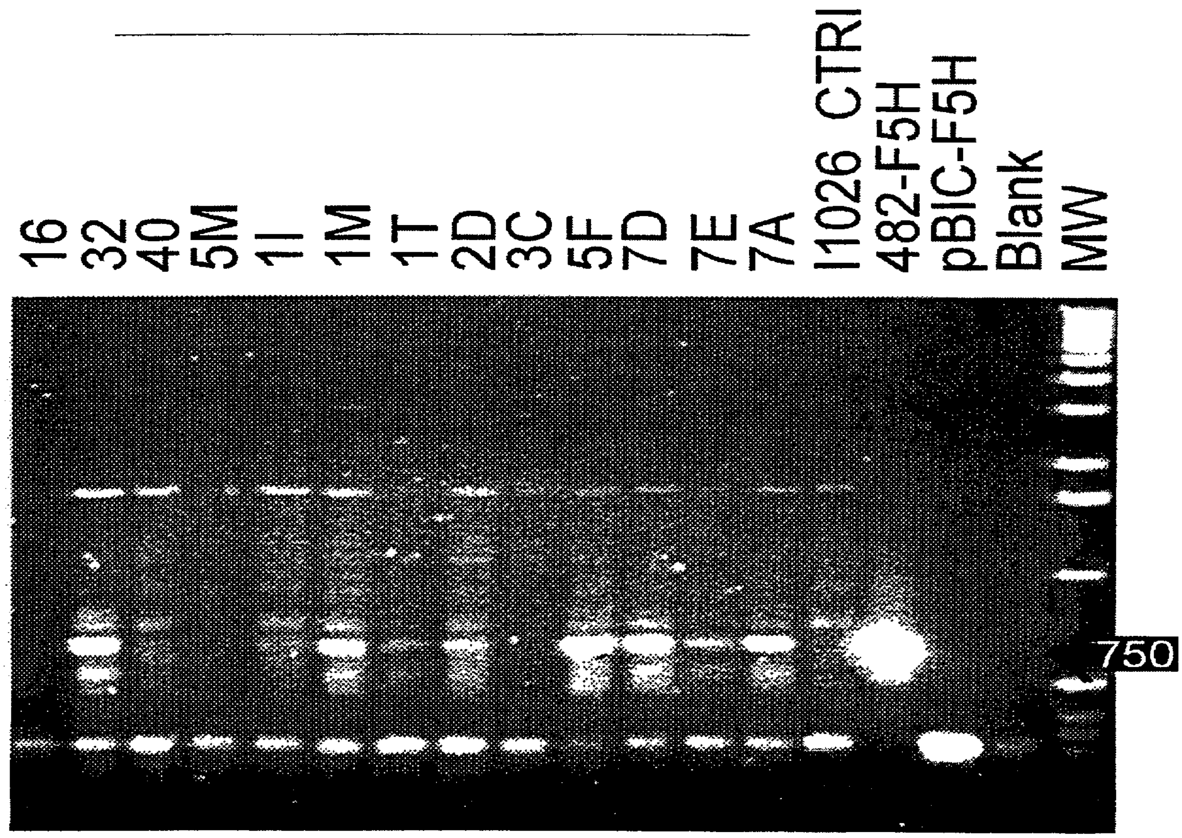
16
32
40
5M
1I
1M
1T
2D
3C
5F
7D
7E
7A
I1026 CTRI
482-F5H
pBIC-F5H
Blank
MW
750

FIG. 3

```
aagcttatgtatttccttataaccattttattctgtatatagggggacagaaacataataagtaacaaatagtggttttattttttttaaa 90
tatacaaaaactgtttaaccattttatttcttggttagcaaaattttgatatattcttaagaaactaatattttaggttgatatattgca 180
gtcactaaatagttttaaaagacacgaagttggtaagaacaggcatatattattcgatttaattaggaatgcttatgttaatctgattcg 270
actaattagaaacgacgatactatgagctcatagatggtcccacgacccactctcccatttgatcaatattcaactgagcaatgaaacta 360
attaaaaacgtggttagattaaaaaaataaattgtgcaggtagcggatatataatactagtaggggttaaaaataaaataaaacaccaca 450
gtattaaattttttgtttcaaaagtattatcaatagtttttttgcttcaaaaatatcacaaattttttgtatgaaatatttctttaacgaaa 540
ataaattaaataaaatttaaaatttatatttggagttctatttttaatttagagtttttattgttaccacatttttttgaattattctaat 630
attaatttgtgatattattacaaaaagtaaaaatatgatattttagaatactattatcgatatttgatattattgaccttagctttgttt 720
gggtggagacatgtgattatcttattacctttttattccatgaaactacagagttcgccaggtaccatacatgcacacaccctcgtgaag 810
ccgtgacttaatatgatctagaacttaaatagtactactaattgtgtcatttgaactttctcctatgtcggtttcacttcatgtatcgca 900
gaacaggtggaatacagtgtccttgagtttcacccaaatcggtccaattttgtgatatatattgcgatacagacatacagcctacagagt 990
tttgtcttagcccactggttggcaaacgaaattgtctttattttttttatgttttgttgtcaatgtgtctttgtttttaactagattgagg 1080
tttaattttaatacatttgttagtttacagattatgcagtgtaatctgataatgtaagttgaactgcgttggtcaaagtcttgtgtaacg 1170
cactgtatctaaattgtgagtaacgacaaaataattaaaattaaaggaccttcaagtattattagtatctctgtctaagatgcacaggta 1260
ttcagtaatagtaataaataattacttgtataattaatatctaattagtaaaccttgtgtctaaacctaaatgagcataaatccaaaagc 1350
aaaaatctaaacctaactgaaaaagtcattacgaaaaaaagaaaaaaaaaagagaaaaaactacctgaaaagtcatgcacaacgttcatc 1440
ttggctaaatttatttagtttattaaatacaaaaatggcgagtttctggagtttgttgaaaatatatttgtttagccactttagaatttc 1530
ttgttttaatttgttattaagatatatcgagataatgcgtttatatcaccaatattttttgccaaactagtcctatacagtcatttttcaa 1620
cagctatgttcactaatttaaaacccactgaaagtcaatcatgattcgtcatatttatatgctcgaattcagtaaaatccgtttggtata 1710
ctatttatttcgtataagtatgtaattccactagatttccttaaactaaattatatatttacataattgttttctttaaaagtctacaac 1800
agttattaagttataggaaattatttcttttatttttttttttttttaggaaattatttcttttgcaacacatttgtcgtttgcaaactt 1890
ttaaaagaaaataaatgattgttataattgattacatttcagtttatgacagatttttttttatctaacctttaatgtttgtttccctgtt 1980
tttaggaaaatcataccaaaatatatttgtgatcacagtaaatcacggaatagttatgaccaagattttcaaagtaatacttagaatcct 2070
attaaataaacgaaattttaggaagaaataatcaagattttaggaaacgatttgagcaaggatttagaagatttgaatctttaattaaat 2160
attttcattcctaaataattaatgctagtggcataatattgtaaataagttcaagtacatgattaatttgttaaaatggttgaaaaatat 2250
atatatgtagatttttttcaaaaggtatactaattattttcatattttcaagaaaatataagaaatggtgtgtacatatatggatgaagaa 2340
atttaagtagataatacaaaaatgtcaaaaaaagggaccacacaatttgattataaaacctacctctctaatcacatcccaaaaTGGAGA 2430
ACTTTGCCTCCTGACAACATTTCAGAAAATAATCGAATCCAAAAAAAACACTCAATATGGAGTCTTCTATATCACAAACACTAAGCAAAC 2520
                                                                 M  E  S  S  I  S  Q  T  L  S  K
TATCAGATCCCACGACGTCTCTTGTCATCGTTGTCTCTCTTTTCATCTTCATCAGCTTCATCACACGGCGGCGAAGGCCTCCATATCCTC 2610
L  S  D  P  T  T  S  L  V  I  V  V  S  L  F  I  F  I  S  F  I  T  R  R  R  R  P  P  Y  P
CCGGTCCACGAGGTTGGCCCATCATAGGCAACATGTTAATGATGGACCAACTCACCCACCGTGGTTTAGCCAATTTAGCTAAAAAGTATG 2700
P  G  P  R  G  W  P  I  I  G  N  M  L  M  M  D  Q  L  T  H  R  G  L  A  N  L  A  K  K  Y
GCGGATTGTGCCATCTCCGCATGGGATTCCTCCATATGTACGCTGTCTCATCACCCGAGGTGGCTCGACAAGTCCTTCAAGTCCAAGACA 2790
G  G  L  C  H  L  R  M  G  F  L  H  M  Y  A  V  S  S  P  E  V  A  R  Q  V  L  Q  V  Q  D
GCGTCTTCTCGAACCGGCCTGCAACTATAGCTATAAGCTATCTGACTTACGACCGAGCGGACATGGCTTTCGCTCACTACGGACCGTTTT 2880
S  V  F  S  N  R  P  A  T  I  A  I  S  Y  L  T  Y  D  R  A  D  M  A  F  A  H  Y  G  P  F
GGAGACAGATGAGAAAAGTGTGTGTCATGAAGGTGTTTAGCCGTAAAAGAGCTGAGTCATGGGCTTCAGTTCGTGATGAAGTGGACAAAA 2970
W  R  Q  M  R  K  V  C  V  M  K  V  F  S  R  K  R  A  E  S  W  A  S  V  R  D  E  V  D  K
TGGTCCGGTCGGTCTCTTGTAACGTTGGTAAgctacttcacatattcaccactcttgctatatatatgtgcaattaaacaaatatgtaaa 3060
M  V  R  S  V  S  C  N  V  G  K
aagtgaaagtactcatttcttctttctttagtatgtactttaacatttaaccaaaacaattgtaggtaagCCTATAAACGTCGGGGAGCA 3150
                                                                          P  I  N  V  G  E  Q
AATTTTTGCACTGACCCGCAACATAACTTACCGGGCAGCGTTTGGGTCAGCCTGCGAGAAGGGACAAGACGAGTTCATAAGAATCTTACA 3240
  I  F  A  L  T  R  N  I  T  Y  R  A  A  F  G  S  A  C  E  K  G  Q  D  E  F  I  R  I  L  Q
AGAGTTCTCTAAGCTTTTTGGAGCCTTCAACGTAGCGGATTTCATACCATATTTCGGGTGGATCGATCCGCAAGGGATAAACAAGCGGCT 3330
  E  F  S  K  L  F  G  A  F  N  V  A  D  F  I  P  Y  F  G  W  I  D  P  Q  G  I  N  K  R  L
CGTGAAGGCCCGTAATGATCTAGACGGATTTATTGACGATATTATCGATGAACATATGAAGAAGAAGGAGAATCAAAACGCTGTGGATGA 3420
  V  K  A  R  N  D  L  D  G  F  I  D  D  I  I  D  E  H  M  K  K  K  E  N  Q  N  A  V  D  D
TGGGGATGTTGTCGATACCGATATGGTTGATGATCTTCTTGCTTTTTACAGTGAAGAGGCCAAATTAGTCAGTGAGACAGCGGATCTTCA 3510
  G  D  V  V  D  T  D  M  V  D  D  L  L  A  F  Y  S  E  E  A  K  L  V  S  E  T  A  D  L  Q
AAATTCCATCAAACTTACCCGTGACAATATCAAAGCAATCATCATGGtaattatatttcaaaaagcactagtcatagtcatgtttcttaa 3600
  N  S  I  K  L  T  R  D  N  I  K  A  I  I  M
tgcgttacgtaataatacttatccattgaccagttattttctcctaagtttttttgtttgaattaggaaggtaattttctattttactag 3690
agaaagcaacagatttttagcatgatctttttttaatatatatagaagcattgaatattcagatctacaataattatgaaactaatgaaga 3780
gacaaaaaatggagagagaaaaaagaaagagtggactagtgtggatatatttaattctaatttgattttattaggacgttatatttaatt 3870

ctaatttgatttttttatttgattttattaggACGTTATGTTTGGAGGAACGGAAACGGTAGCGTCGGCGATAGAGTGGGCCTTAACGGA 3960
                                 D  V  M  F  G  G  T  E  T  V  A  S  A  I  E  W  A  L  T  E
GTTATTACGGAGCCCCGAGGATCTAAAACGGGTCCAACAAGAACTCGCCGAAGTCGTTGGACTTGACAGACGAGTTGAAGAATCCGACAT 4050
  L  L  R  S  P  E  D  L  K  R  V  Q  Q  E  L  A  E  V  V  G  L  D  R  R  V  E  E  S  D  I
CGAGAAGTTGACTTATCTCAAATGCACACTCAAAGAAACCCTAAGGATGCACCCACCGATCCCTCTCCTCCTCCACGAAACCGCGGAGGA 4140
  E  K  L  T  Y  L  K  C  T  L  K  E  T  L  R  M  H  P  P  I  P  L  L  L  H  E  T  A  E  D
CACTAGTATCGACGGTTTCTTCATTCCCAAGAAATCTCGTGTGATGATCAACGCGTTTGCCATAGGACGCGACCCAACCTCTTGGACTGA 4230
  T  S  I  D  G  F  F  I  P  K  K  S  R  V  M  I  N  A  F  A  I  G  R  D  P  T  S  W  T  D
CCCGGACACGTTTAGACCATCGAGGTTTTTGGAACCGGGCGTACCGGATTTCAAAGGGAGCAATTTCGAGTTTATACCGTTCGGGTCGGG 4320
  P  D  T  F  R  P  S  R  F  L  E  P  G  V  P  D  F  K  G  S  N  F  E  F  I  P  F  G  S  G
TCGTAGATCGTGCCCGGGTATGCAACTAGGGTTATACGCGCTTGACTTAGCCGTGGCTCATATATTACATTGCTTCACGTGGAAATTACC 4410
  R  R  S  C  P  G  M  Q  L  G  L  Y  A  L  D  L  A  V  A  H  I  L  H  C  F  T  W  K  L  P
TGATGGGATGAAACCAAGTGAGCTCGACATGAATGATGTGTTTGGTCTCACGGCTCCTAAAGCCACGCGGCTTTTCGCCGTGCCAACCAC 4500
  D  G  M  K  P  S  E  L  D  M  N  D  V  F  G  L  T  A  P  K  A  T  R  L  F  A  V  P  T  T
GCGCCTCATCTGTGCTCTTTAAGTTTATGGTTCGAGTCACGTGGCAGGGGGTTTGGTATGGTGAAAACTGAAAAGTTTGAAGTTGCCCTC 4590
  R  L  I  C  A  L  *
ATCGAGGATTTGTGGATGTCATATGTATGTATGTGTATACACGTGTGTTCTGATGAAAACAGATTTGGCTCTTTGTTTGCCCTTTTTTTT 4680
TTTTTCTTTAATGGGGATTTTCCTTGAATGAAATGTAACAGTAAAAATAAGATTTTTTTCAATAAGTAATTTAGCATGTTGCaaagatcg 4770
atcttggatgagaacttctacttaaaaaaaaaaaaaaattttttttagttatttcaccttttttcttttgttctggttgtatggttgcc 4860
attgtgtcaattaggggctggaagttcgctggttaaggctaaatcagagttaaagttataatttacaagcccaacaaaaggtcgcagat 4950
taaaaccacatgatatttataaaaaaaattctaaggttttattagttttattttcagtttactgagtactatttactttttttattttt 5040
gcaaataaatgtattttatcatatttatgtttttttgttataaactccaaacatacaggtttcattacctaaaaaaagacagagtggtttc 5130
gttaattttgtttcattaatctcgag 5220
```

Fig. 4

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	205502-9004
First Named Inventor	ELLIS, David Dunham
COMPLETE IF KNOWN	
Application Number	PCT/CA00/00074
Filing Date	31 JAN 2000
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MODIFICATION OF LIGNIN COMPOSITION OF GYMNOSPERMS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 31 JAN 2000 as United States Application Number or PCT International

Application Number PCT/CA00/00074 and was amended on (MM/DD/YYYY) ______ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
PCT/CA00/00074	CANADA	01/31/2000	☐	☐	☐
60 / 118,124	US	01 FEB 1999	☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION — Utility or Design Patent Application

Direct all correspondence to: [ ] Customer Number or Bar Code Label [ ] OR [X] Correspondence address below

Name MICHAEL BEST & FRIEDRICH LLC			
Address 401 N. Michigan Avenue, Suite 1700			
City Chicago		State Illinois IL	ZIP 60611
Country U.S.A.	Telephone (312)661-2100		Fax (312)661-0029

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR :	[ ] A petition has been filed for this unsigned inventor		
1 Given Name (first and middle [if any]) David Dunham		Family Name or Surname ELLIS	
Inventor's Signature [signature]			Date Nov 27, 2001
Residence: City Tsawwassen	State B.C.	Country CAN Canada	Citizenship
Mailing Address 4887 5th Avenue			
City Tsawwassen	State B.C.	ZIP V4M 1J5	Country Canada

NAME OF SECOND INVENTOR:	[ ] A petition has been filed for this unsigned inventor		
2 Given Name (first and middle [if any]) Clinton Charles Spencer		Family Name or Surname CHAPPLE	
Inventor's Signature			Date
Residence: City West Lafayette	State Indiana IN	Country USA	Citizenship
Mailing Address 2210 Robinhoold Lane			
City West Lafayette	State Indiana	ZIP 47906-5029	Country USA

[X] Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (11-00)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION	ADDITIONAL INVENTOR(S) Supplemental Sheet Page 3 of 3

30

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Margarita	GILBERT

Inventor's Signature M Gilbert		CH	Date NOV. 27, 2001
Residence: City Maple Ridge	State B.C.	Country Canada	Citizenship
Mailing Address #38-23085 118 Avenue			
Mailing Address			
City Maple Ridge	State B.C.	ZIP V2X 3J7	Country CANADA

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature			Date
Residence: City	State	Country	Citizenship
Mailing Address			
Mailing Address			
City	State	ZIP	Country

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature			Date
Residence: City	State	Country	Citizenship
Mailing Address			
Mailing Address			
City	State	ZIP	Country

PTO/SB/01 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION — Utility or Design Patent Application

Direct all correspondence to: ☐ Customer Number or Bar Code Label ______ OR ☒ Correspondence address below

Name: MICHAEL BEST & FRIEDRICH LLC

Address: 401 N. Michigan Avenue, Suite 1700

City: Chicago | State: Illinois | ZIP: 60611

Country: U.S.A. | Telephone: (312)661-2100 | Fax: (312)661-0029

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR : ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]): David Dunham | Family Name or Surname: ELLIS

Inventor's Signature: | Date:

Residence: City: Tsawwassen | State: B.C. | Country: Canada | Citizenship:

Mailing Address: 4887 5th Avenue

City	State	ZIP	Country
Tsawwassen	B.C.	V4M 1J5	Canada

NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]): Clinton Charles Spencer | Family Name or Surname: CHAPPLE

Inventor's Signature: Clinton Charles Spencer Chapple | Date: Feb. 4, 2002

Residence: City: West Lafayette | State: Indiana | Country: USA | Citizenship:

Mailing Address: 2210 Robinhoold Lane

City: West Lafayette | State: Indiana | ZIP: 47906-5029 | Country: USA

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.